

STUDIES ON THE RELEASE OF ENDOGENOUS RENAL DOPAMINE
AND ASSESSMENT OF THE RENAL DOPAMINE PRODRUG GLUDOPA
IN NORMAL MAN

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Papers resulting from work in this thesis

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Declaration

The studies in this thesis were designed and undertaken by myself and the composition is my own. Contributions and assistance from others are clearly acknowledged. The thesis has not been submitted in candidature for any other degree, diploma or professional qualification.

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CHAPTER ONE

INTRODUCTION

1) SALT BALANCE AND MECHANISMS OF NATRIURESIS

The sodium ion occupies a unique and central role in animal function. Sodium salts, in particular sodium chloride, comprise more than 90% of the total osmoles in the extracellular fluid space (ECF). Active extrusion from the intracellular space renders sodium principally an extracellular ion, whereas water moves readily between the compartments along osmotic gradients. Plasma osmolality is closely regulated within narrow limits and it thus follows that the volume of the ECF is determined by its total osmolar content.

Normal tissue function requires that blood is delivered under optimal perfusion pressures and flow rates. A principle determinant of these is the ECF and particularly the plasma volume, and it would seem logical that animals have developed powerful and complex mechanisms to maintain ECF volume within narrow limits.

Sodium homeostasis results from a balance between dietary intake and excretion. The body contains some 4000 mmols of sodium and nearly all of the exchangeable sodium resides in the extracellular compartment. There is limited ability to store sodium within the body so that extremely tight control of excretion must exist during periods of salt scarcity. Salt thirst is well recognised in animals and Denton (1982) has reviewed evidence for a similar phenomenon in man. As for sodium excretion, faecal losses of sodium are poorly controlled, but acclimatisation to severe environmental conditions does involve reduction of salt loss in sweat (Conn, 1949). However, the kidney remains the principle determinant of salt excretion and sodium balance and any discussion on homeostatic control of sodium and ECF volume must focus on the primacy of this organ.

In large areas of the planet, sodium is scarce and it is present in only

trace amounts in most plants. Accordingly, during evolutionary development for a wide variety of animals, in various ecological niches, there would be great survival value in the possession of effective mechanisms for the acquisition of salt and of its retention in the body. Anthropological evidence suggests that the development of hominoids over 30 million years from the late Oligocene period has been determined by pressures operating to influence salt retaining mechanisms. Up to the late Pliocene time, diet was predominantly if not exclusively vegetarian. Early man (1.7 million years) was a hunter of large animals. However it is likely that meat made up a small and intermittent fraction of the diet. Clark (1970) emphasises that vegetarian foods form 60-80% of the diet of hunter-gatherers in warm and temperate climates today, the proportion decreasing as one approaches the poles. Some contemporary feral societies remain almost entirely vegetarian today. New Guinea Highlanders have a diet of which sweet potatoes form the basis. Analysis of urine excretion suggests a daily ingestion of only 1-3 mmol of sodium per day with a K^+/Na^+ ratio of up to 400 (Oomen et al., 1961). Similarly, the Chimbu tribe of New Guinea and Yanomamö Indians of Tropical Venezuela and Brazil have predominantly vegetarian diets with very low salt intakes (MacFarlane et al., 1964; Oliver et al., 1975). The low sodium status is no physiological handicap, but is reflected in very high aldosterone levels.

Thus man has developed in salt-poor circumstances and has enjoyed successful existence due to the development of avid salt-retaining responses. Tropical regions are extremely poor in salt, most sodium being present in animals. Denton (1982) has drawn attention to this and put forward the provocative contention that endocannibalism, seemingly widely practiced until recently, and setting aside the religious symbolism, was in fact evolutionarily advantageous in allowing recirculation of a scarce mineral.

Over the last 100 years or so, dietary content has changed dramatically in many parts of the world. From being highly sought after, prized and expensive, salt has, in the developed world, become cheap and abundant. Sodium intake has markedly increased so that 'normal' intake in the USA, Europe and Australia is now 50-400 mmols per day and in some areas such as northern Honshu in Japan, the figure is upwards of 500 mmol/day. Interest is now focused on the manner by which the kidneys are able to excrete such supraphysiological amounts of salt. This is not just an academic question. The incidence of hypertension is exceedingly high in Japan and other countries where salt intake is high whereas it is almost unknown in those communities with very low sodium intakes. It has been hypothesised that some individuals have a genetically determined inability to excrete salt adequately and that renal salt retention is a causative factor in the development of high blood pressure (de Wardener and MacGregor, 1982). There is interest in differential sensitivity of different populations to high salt intake. It is well known that Negro populations in the USA are more prone to hypertension and that they suffer greater morbidity and mortality from its effects than American Caucasians (Akinkugbe, 1985). Desor et al. (1975) have shown that black children have greater preference for the salt taste. In addition, Negroes have reduced capacity to excrete an intravenously administered salt load, in comparison with whites (Luft et al., 1977). Whites reached the USA after migration from Europe and 20-40,000 generations in temperate regions. They lost skin pigment as an evolutionary adaptation and it may be that changes in sodium control also occurred. In the cooler climate, there would have been reduced sodium losses from skin and amelioration of pressure on sodium homeostasis. Blacks arrived in the USA only 200-400 years ago. Thus their history is in the tropics with recent considerable salt stress. They may have less capacity to excrete salt and greater hedonic

preference.

Strauss et al. (1958) conducted classical balance studies in man which have been confirmed repeatedly in animals and man. If an individual is in balance on a 10 mmol sodium diet and this is increased to 150 mmol, sodium excretion will increase until, after 3-5 days or so, output again matches intake. However, there will be a period of initial sodium retention and the new steady state will be reached with a small increase in weight, exchangeable sodium, plasma and ECF volumes and venous pressure. The reverse set of events occurs when salt intake is again reduced. Thus the body functions in a homeostatic manner, in that marked changes in input are detected and the body's response is to correct for any changes, keeping total body sodium within narrow limits. As in all homeostatic mechanisms, salt balance is achieved by a feedback loop principle with an afferent or sensing mechanism, feeding information to a central processor, which then determines the effector response. Implicit in such a system, is a 'set point' around which balance occurs and towards which control mechanisms operate. This concept has received relatively little attention, but Hollenberg (1980) has addressed the question. He defines 'set point' as that amount of sodium chloride in the body when the individual is in balance on zero salt intake (or at least allowing for small obligatory losses in urine, faeces, skin). Thus an individual on a 10 mmol sodium diet will promptly excrete an extra salt load, even as little as 30 mmol. But if a diuretic is first given resulting in loss of some 100 mmols, then gaining 30 mmol will not result in a natriuresis. Only when the deficit of 100 mmol is replaced and the body returned above zero set point will a natriuresis occur after salt addition. The studies with primitive feral communities ingesting less than 5 mmol/day show individuals to be perfectly healthy. According to the above theory, they are not to be considered in salt deficit or negative sodium

balance. However it does indicate that any metabolic stress such as diarrhoeal illness may rapidly move individuals in such populations into severe sodium deficit.

As stated above, the kidney has the important function of regulating salt excretion commensurate with requirements. In addition, the kidney must regulate other ions, acid-base and water balance, and excrete waste products of metabolism. The kidney is able to perform such diverse functions by means of morphological and functional nephron heterogeneity and by operating against a background of enormous turnover. Over one fifth of the cardiac output is to the kidneys and each day 25,000 mmol of sodium are filtered of which around 99% are reabsorbed. It will be apparent that only small alterations in renal control can result in very large changes in sodium excretion.

The last two decades have seen remarkable advances in our knowledge of renal function. Fields such as glomerular and tubular dynamics, electrophysiology, hormone and drug receptor interactions, tubule transport functions, and molecular biochemistry and enzymology have been extended and enlarged by refinement of traditional methodology and as a consequence of new in vivo and vitro techniques.

Much research seeks to dissect out particular stimulus and response characteristics, free from extraneous and interacting influences. A challenge, particularly for in vitro studies, is to identify those phenomena which are physiologically relevant. Precaution is also required in interpreting results in whole animal or human studies where responses are evoked by stimuli unlikely to occur in nature.

Afferent Mechanisms

As already stated, maintenance of ECF volume is an important homeostatic function, requiring information on the fullness of the circulation or "effective circulating volume", before responses can be initiated. As befitting such an important function, there is evidence that volume detectors reside on venous and arterial sides of the systemic circulation and in the kidney itself.

Neural receptors, comprising branching ends of small medullated fibres running in the vagus nerve, have been identified in the atria and central compliance veins, and shown to alter discharge with mechanical stretch or changes in transmural pressure. Various manoeuvres resulting in changes in venous return and central venous pressure, such as adoption of the supine position, weightlessness, negative-pressure breathing and head-out water immersion, are associated with an acute natriuretic response. Many workers have demonstrated increased sodium output following left atrial distension produced by balloon inflation in dogs. Interpretation of latter findings has been rendered more complex by the discovery of atrial natriuretic peptide which will be discussed later. However, at least one study has demonstrated that the natriuresis of atrial stretch was completely abolished by cervical vagotomy. It should be pointed out however, that not all groups concur with an important role for intrathoracic volume receptors in sodium homeostasis. A number of studies have demonstrated either trivial, or no changes in sodium excretion, following cardiac denervation or cervical vagotomy, in response to volume expansion (reviewed by Reineck et al., 1985; Seifter et al., 1986).

That volume receptors exist on the arterial side of the circulation was first inferred by Epstein et al (1953). They demonstrated that closure of post-traumatic atrio-ventricular fistulae in Korean War Veterans resulted in a

natriuresis, concomitant with an increase in diastolic pressure and decrease in right atrial and central venous pressures. The site of the arterial receptor is unknown, but the carotid sinus baroreceptor has been proposed as a likely site. Traction stimulation of the baroreceptor in anaesthetised rats resulted in a large natriuresis (Keeler, 1974) and two groups have shown that bilateral carotid ligation is antinatriuretic (Zambraski et al., 1976; Di Bona, 1977). The reflex response was shown to be dependant on intact renal innervation and bilateral carotid ligation was shown to increase renal sympathetic nerve activity.

Afferent fibres from volume receptors are believed to travel along the IX and X cranial nerves to hypothalamic and medullary centres. From here integrated physiological responses are initiated including antidiuretic hormone release and modulation of sympathetic nerve discharge to the periphery. In addition, it seems likely that intracranial receptors exist with a role in salt balance: injection of hypertonic saline into the third or fourth ventricle directly increases sodium output (Passo et al., 1975). A role for the brain in determining the balance between natriuretic and antinatriuretic mechanisms has been given further credence by the demonstration of receptor sites for atrial natriuretic peptide and angiotensin II. In addition, the hypothalamus is believed to produce the putative natriuretic hormone, Na^+/K^+ -ATPase inhibitor (vide infra).

That the kidney itself is capable of sensing changes in intravascular volume or pressure has been demonstrated using the isolated perfused whole kidney preparation. Increased perfusion pressure promotes a natriuretic response by virtue of a baroreceptor mechanism at the juxtaglomerular apparatus. The efferent response involves modulation of the activity of the hormonal renin-angiotensin system which will be discussed later.

Efferent Mechanisms

As indicated, the afferent sensing component of body sodium control is organised at a number of sites to allow an integrated response. Similarly, it is evident that efferent mechanisms are multiple and originate both within and outside the kidney, although the kidney is the final common pathway for control. Salt excretion is determined by a complex interaction of neuronal, hormonal and physical factors and many mechanisms have been defined and demonstrated to alter salt excretion. What is not clear always, is the relative importance of each, and its physiological interaction with others under normal conditions.

Renal Nerves

The postganglionic innervation of the kidneys is composed mainly of adrenergic vasomotor fibres. Histochemical staining for catecholamines reveals an extensive adrenergic innervation of all arterial components, particularly concentrated at the afferent arterioles in the region of the juxtaglomerular apparatus (McKenna and Angelakos, 1968). More recently, Müller and Barajas (1972) using electronmicroscopy and histochemical techniques, have demonstrated adrenergic innervation of proximal and distal tubules in monkey and rat. The result of changes in volume status of the body is modulation of efferent sympathetic outflow to the periphery including the kidneys. Thus Prosnitz and Di Bona (1978) have demonstrated in the dog that an increase of 15 mmHg in left atrial pressure reduced renal nerve activity by 40% and increased sodium excretion by 80%. Bilateral cervical vagotomy abolished both the decrease in

renal nerve activity and the natriuresis. Along these lines, Thames et al. (1982) have reported a significant inverse relationship between pulmonary artery wedge pressure and renal nerve activity.

The phenomenon of denervation-natriuresis has been long recognised as has the acute reduction in sodium output produced by renal nerve stimulation. However, almost all early studies were performed on anaesthetised animals and Smith (1951), repeating studies on conscious dogs, was unable to demonstrate any effect of denervation on salt output. The prevailing view is that the renal nerves exert little tonic influence under normal circumstances and that denervation-natriuresis is an artifact of anaesthesia-induced renal vasoconstriction (reviewed by Gottschalk et al., 1985). Some studies however, have suggested that renal nerves have a physiological role under certain circumstances. Thus Schneider et al. (1978) demonstrated that renally denervated dogs are unable to appropriately conserve sodium when maintained on a low salt diet. Patients with idiopathic autonomic insufficiency syndrome are assumed to have partial or complete renal denervation. Bartter et al. (1959) found that such patients were also unable to decrease urinary sodium excretion appropriately when placed on a salt restricted diet.

The renal nerves can potentially modulate sodium excretion by more than one mechanism. Direct nerve stimulation can produce large decrements in glomerular filtration rate (GFR) and renal blood flow (RBF) with stimulation of renin release being contributory. More recent studies however, with low level electrical nerve stimulation have shown reduced sodium excretion in the absence of haemodynamic changes. That this is due to a direct effect at the proximal tubule has been confirmed by micropuncture studies (reviewed by Raymond and Stein, 1987). Although renal nerve stimulation results in activation of both the renin-angiotensin and prostaglandin systems, both of which may

affect sodium balance, Zambraski and Di Bona (1976; 1982) showed that blockade of both systems, with saralasin and indomethacin, did not attenuate the effect of renal nerve stimulation. There is little dispute that renal vasoconstriction is mediated by an effect at α -adrenergic receptors on the vasculature. However, the receptor subtype subserving the tubular effect is not clear and in vivo micropuncture and in vitro microperfusion of proximal tubules have been utilised to address this question. Although some reports suggest that the β -receptor is of importance, most studies indicate that the α -receptor mediates the increased sodium reabsorption induced by renal nerve stimulation (reviewed by Kim et al., 1980).

Glomerular filtration rate and glomerular-tubular balance

With control of sodium excretion taking place against a background of enormous turnover it might be expected that small alterations in GFR would elicit significant alterations in sodium excretion. In fact, modulation of tubular reabsorption ensures relative constancy of fractional sodium excretion. This coupling between filtration and tubular reabsorption is termed glomerular-tubular balance and has been confirmed in whole animal studies, in the isolated perfused kidney preparation and by micropuncture (reviewed by Reineck et al., 1985).

To appreciate this phenomenon requires understanding of processes controlling sodium reabsorption in the nephron. Most studies have focused on the part played by the proximal tubule though other segments of the nephron contribute to sodium homeostasis.

Reabsorption of fluid from the proximal tubule is isosmotic and ultimately depends upon the active transport of sodium across the basolateral membrane of the proximal tubular cells, mediated by the enzyme Na^+/K^+ -ATP

ase. Sodium enters the cells from the lumen down electrochemical gradients and its passive transfer across the apical membrane is linked to ion and solute transport in electrogenic or neutral processes. 50-70% of sodium transport occurs in the proximal tubule underlying its importance and capacity for control. Uptake of fluid into the peritubular capillary is dependant on the passive Starling forces governing transport across all capillaries. Fluid flux can be expressed by the arithmetical formula:

$$\text{flux} = K_f (\Delta P - \Delta \pi)$$

where ΔP is the hydrostatic pressure gradient across the capillary, $\Delta \pi$ is the oncotic pressure gradient and K_f an ultrafiltration coefficient. At the peritubular capillary, forces favour reabsorption, and it is evident that the rate of uptake is dependant on the peritubular capillary oncotic pressure and hydrostatic pressure, and the plasma flow rate which determines the rate of delivery of oncotically active particles. The peritubular oncotic pressure is believed to be of particular importance, and by means of micropuncture, Brenner et al. (1969a) have clearly demonstrated a direct relationship between proximal reabsorption and the peritubular oncotic pressure. Under circumstances in which GFR alters without change in renal haemodynamics, peritubular capillary oncotic pressure will vary with the filtration fraction, that is, the fraction of plasma water extracted by the process of glomerular filtration. The result will be the maintenance of glomerular-tubular balance. The passive Starling forces are not incompatible with the active nature of sodium reabsorption across the proximal tubule. The explanation lies in the so called "pump-leak" model of reabsorption. Alterations in the physical forces will influence the rate of uptake of reabsorbate from the interstitial space surrounding the tubule. If the balance of forces does not favour uptake, and interstitial pressure increases, fluid will leak back into the lumen across the tight junctions (Reineck et al.,

1985). The mutual interdependence of active and passive processes was demonstrated by Green et al. (1974) who showed that in the absence of active sodium transport, peritubular oncotic pressure failed to exert an influence on proximal reabsorption.

One of the first studies to demonstrate disruption of glomerular-tubular balance was the work of de Wardener and colleagues (1961). They demonstrated in the dog that volume expansion with saline produced an acute natriuresis even when GFR and RBF were artificially reduced by aortic clamping. It has been shown by micropuncture that saline loading causes a reduction in proximal fractional sodium reabsorption (Dirks et al., 1965).

Ichikawa and Brenner (1979) have further studied this phenomenon, drawn attention to the importance of the change in plasma protein concentration during intravenous salt loading, and stressed peritubular forces. Thus Stein et al. (1973) have revealed greater increases in sodium excretion in saline-expanded rats than in those with equivalent plasma expansion produced by hyperoncotic albumin infusions. Intravenous saline loading, producing measurable changes in plasma oncotic pressure, is unlikely to be a physiological manoeuvre. However, under physiological conditions, physical forces are still likely to be important. Alterations in renal haemodynamics produce disruption of glomerular-tubular balance through changes in the filtration fraction. At any given plasma protein concentration, the magnitude of the oncotic force across the peritubular capillary is in large part determined by the filtration fraction. This parameter is in turn influenced by efferent arteriolar tone, which modulates both peritubular capillary oncotic and hydrostatic pressures. Manoeuvres which increase renal blood flow (pharmacological vasodilators, increased salt diet, hormones) usually reduce the filtration fraction, the effect of which is to discourage reabsorption and increase sodium excretion. The

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physical forces at the proximal tubule are thought to mediate, at least in part, the phenomenon of pressure natriuresis (Koch et al., 1968).

Neuronal and hormonal influences can modulate the effects of physical factors. Thus the renal nerves and angiotensin II have direct effects at the proximal tubule, but in addition they effect changes in renal haemodynamics and influence the filtration-fraction.

Sodium transport across more distal segments of the nephron has not been studied in as great detail as that across the proximal tubule, principally because deep segments are not amenable to micropuncture. The role of other segments of the nephron in the response to salt loading and volume expansion is therefore not entirely clear. Most parts of the nephron can be shown to exhibit volume or load dependancy of reabsorption, a process that will tend to maintain glomerular-tubular balance. Thus sodium excretion in the urine is normally less than the delivery to the distal nephron produced by reduction of proximal reabsorption. Stein et al (1976) have provided some evidence that the thin ascending limb of the loop of Henlé in juxtamedullary nephrons participates in the natriuretic response to volume expansion. The mechanism involves an increase in medullary blood flow allowing dissipation of the hypertonic interstitium, which normally encourages passive reabsorption of sodium from the ascending limb (Reineck et al., 1985). In addition, as will be presented later, the distal tubule appears to be the site of action of a number of hormones involved in salt homeostasis.

Renin-angiotensin-aldosterone axis

Tigerstedt and Bergman partially purified a pressor substance from freshly excised rabbit kidney in 1898. They named the agent renin and since that time a voluminous literature has accumulated on this antinatriuretic hor-

monal axis. It forms a complex, interacting system, a principle function of which is the maintenance of glomerular filtration rate and sodium balance under conditions of sodium depletion and reduction in renal perfusion pressure. Renin is secreted in the kidney from juxtaglomerular cells, differentiated myo-epithelial cells located at the distal end of the afferent arteriole. This region is in apposition to specialised cells of the distal tubule comprising the macula densa. A great number of inputs have been reported to influence renin secretion but three stimuli in particular are accepted as physiologically important (reviewed by Davis and Freeman, 1976).

1. A reduction in renal perfusion pressure stimulates renin secretion mediated by an intrarenal baroreceptor believed to reside at the juxtaglomerular cells.
2. Sympathetic nerve stimulation and circulatory catecholamines increase renin release via β -adrenergic receptors on the juxtaglomerular cells.
3. The macula densa is somehow able to sense the composition of distal tubular fluid. Thus, a reduction in sodium and/or chloride load or concentration stimulates renin secretion.

In addition, angiotensin II produces feedback inhibition of renin release (Vander and Geelhoed, 1965), and the prostaglandins are also able to modulate renin release (*vide infra*).

Renin, a proteolytic enzyme, acts on a circulating α_2 globulin, angiotensinogen, to create a decapeptide angiotensin I. The latter has little or no activity until metabolised by a converting enzyme to the octapeptide angiotensin II. Hydrolysis of angiotensin II yields the heptapeptide angiotensin III which has similar activity, but a shorter biological half-life (Koushanpour and Kriz, 1986).

It was widely believed that the principle function of angiotensin II was in stimulation of aldosterone release from the adrenal cortex (Laragh and Sealey, 1973). However, it is now apparent that angiotensin II itself exerts multiple effects both systemically and in the kidney, and can profoundly affect sodium balance.

Angiotensin II is a potent vasoconstrictor and systemic effects are characterised by changes in peripheral vascular resistance and effective ECF volume, with maintenance of blood pressure. In addition to circulating levels, angiotensin II is produced in the kidney in high concentration where it modulates intrarenal haemodynamics. The renal constrictor action of angiotensin II is confined primarily to the efferent arterioles (Edwards, 1983), the effect of which is to maintain glomerular filtration under circumstances in which renal perfusion may be embarrassed. In addition, the reduction of filtration fraction will, as discussed, by reducing peritubular capillary hydrostatic pressure and increasing capillary oncotic pressure, favour reabsorption of sodium at the proximal tubule (Mujais et al., 1986). Angiotensin II receptors have been identified on proximal tubular cells, and microperfusion studies have demonstrated a direct action in promoting sodium reabsorption at the proximal tubule (Schuster et al., 1984a). The local formation of angiotensin II may contribute to the tubuloglomerular feedback mechanism which relates the composition of fluid at the macula densa to control of GFR (Zanchetti, 1985). There has recently been interest in the action of the renin-angiotensin system in the CNS with the recognition that all components are present within the brain, and there is evidence that this system is independent of the circulating system. Administration of angiotensin II into the cerebral ventricles produces a pressor response in a number of animal models. Part of the response may be through facilitation of sympathetic outflow, and there is evidence that angiotensin

enhances sympathetic nerve activity both centrally and peripherally (Zanchetti, 1985). Thus through multiple mechanisms, angiotensin II maintains glomerular filtration to ensure that processing of products of metabolism can continue, at the same time encouraging retention of sodium within the body to protect ECF volume and blood pressure.

The importance of aldosterone in maintaining sodium balance is evident from the salt wasting and death that occurs in the deficiency state, Addison's disease. Administered aldosterone promotes salt retention and a significant inverse correlation exists between the level of sodium intake and the secretion of the hormone. By micropuncture and isolated tubular perfusion preparations, the site of action of aldosterone has been localised to the cortical collecting tubule where it appears to affect Na^+/K^+ -ATPase (Reineck et al., 1985). Several observations however, indicate that aldosterone is not the primary regulator of urinary sodium excretion and that other factors assume greater importance under certain conditions. In the study of de Wardener et al. (1961) referred to earlier, the natriuretic response to saline loading occurred not only with a reduced GFR, but also in the presence of exogenously administered mineralocorticoid excess. In Addison's disease, normal salt balance can be achieved with a fixed replacement dose of mineralocorticoid, in spite of widely varying dietary salt intake (Rosenbaum et al., 1955). Finally, the chronic administration of mineralocorticoid causes only a transient period of sodium retention after which a natriuresis ensues and balance is restored; the widely studied phenomenon "mineralocorticoid escape" (August et al., 1958).

Prostaglandins

The prostaglandins form a family of compounds produced in many tissues in the body including the kidney, where they function as local hormones or

autacoids (reviewed by Lote and Haylor, 1986; Nadler and Brenner, 1987). The rate limiting step in synthesis is the release of arachidonic acid from membrane bound phospholipids, a process in the kidney stimulated by the vasoactive hormones angiotensin II, noradrenaline, vasopressin and bradykinin. Cyclooxygenase converts arachidonic acid into unstable prostaglandin endoperoxides which are then enzymatically converted to active prostaglandins. The production of various prostaglandins is compartmentalised within the nephron, and the principle sites of synthesis are the cortical arterioles where prostaglandin I_2 (prostacyclin) predominates, and glomerular mesangial and epithelial cells, cortical and medullary collecting ducts, and medullary interstitial cells where prostaglandin E_2 predominates. The prostaglandins have diverse actions within the kidney, effects having been demonstrated on RBF, GFR, renin release and tubular salt and water handling. Many studies have attempted to define physiological roles for renal prostaglandins, but there is a great deal of controversy with discrepant results. The anatomical and functional complexity of the kidney has made evaluation of specific prostaglandin function difficult. In addition, it is likely that prostaglandins act as local regulators of hormonal and other stimuli, so that observed actions of these autacoids will be largely determined by the prevailing influence of other circulating and intrarenal factors.

Four main approaches have been utilised to define a role for prostaglandins in sodium homeostasis; infusion of arachidonic acid or prostaglandins systemically or intrarenally; assessment of endogenous prostaglandin activity under salt depletion and loading manoeuvres; studies into the effect of cyclooxygenase inhibition with non-steroidal anti-inflammatory drugs; and in vitro studies on isolated tubular preparations.

The infusion of arachidonic acid and the major renal prostaglandins PGE_2 and PGI_2 consistently increases RBF and GFR and results in a natriur-

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esis (Ballerman et al., 1986). It is unclear from such studies whether vasodilation completely accounts for the natriuresis or whether a tubular effect can be invoked. The physiological relevance of the doses used is questionable, and it is unlikely that prostaglandins presented in this way will act at the sites at which prostaglandins are endogenously synthesised and utilised.

Manoeuvres to alter sodium status have produced conflicting results. Salt loading has been reported to increase, produce no change, or decrease renal venous and urinary prostaglandin excretion in man and animals (Nielsen et al., 1979; Rathaus et al., 1981; Dunn, 1983). Part of the explanation may be that analysis of prostaglandin content of venous blood or urine provides an overall assessment of excretion, but does not allow for metabolism and compartmentalisation of synthesis, and, in the urine, prostaglandin excretion depends on flow rate.

Studies with cyclo-oxygenase inhibition have produced some consensus concerning the function of renal prostaglandins. In salt replete, unstressed animals and man, inhibition of prostaglandin synthesis produces few ill effects on renal haemodynamics and salt output. Prostaglandins appear to exert an important effect during those conditions in which intrarenal vasoconstrictor factors, angiotensin II and noradrenaline, are increased, such as salt depletion, hypotension, anaesthesia, and surgery. Prostaglandins counteract vasoconstriction of afferent and efferent arterioles to maintain RBF, and under such circumstances cyclo-oxygenase inhibition can result in marked reduction in RBF, GFR and salt excretion (Nadler and Brenner, 1987).

In vitro microperfusion and micropuncture studies have been used to address the question as to whether prostaglandins have direct tubular effects. Although there remains some dispute, it appears that PGE_2 especially, modulates salt transport at the medullary thick ascending loop of Henlé and at the

cortical and medullary collecting ducts. The importance of such effects is not clear (Lote and Haylor, 1986).

Non-steroidal anti-inflammatory drugs reduce plasma renin activity in the baseline state, suggesting that prostaglandins have a physiological role in renin secretion. By the use of various models, an effect of the prostaglandins can be demonstrated on the action of all three of the principle stimuli to renin secretion discussed earlier. However, while the influence of prostaglandins on renin release is established, they are not essential intermediaries, rather they appear to function as modulators of the renin secretory response to other stimuli (Zanchetti, 1985; Lote and Haylor, 1986).

Whether prostaglandins play a role in the natriuretic response to ECF volume loading is not clear. Two groups at least have demonstrated that the natriuresis following saline loading can be blunted by cyclo-oxygenase inhibitors (Higashihara et al., 1979; Wilson et al., 1982), and Carmines et al. (1985) have demonstrated that cyclo-oxygenase inhibition impairs pressure natriuresis. However, such conclusions have not always been confirmed (Kirschenbaum and Stein, 1977).

In summary, the exact role of prostaglandins in volume control has not yet been determined. What seems clear is that the secretion of prostaglandins is tightly coupled to the activity of vasoactive hormones and they function to modulate their renal effects.

Atrial Natriuretic Peptide

As early as 1956 Kisch described the presence of osmophilic granules in guinea pig atrial cardiocytes. This was followed up by Jamieson and Palade (1964), and in 1976, Marie et al. demonstrated that the degree of granularity varied with the water and salt intake of rats. However, the discovery by de

Bold and colleagues (1981) that injection of atrial extracts produced a marked natriuresis in rats, signalled intense interest and a profusion of research into a new putative natriuretic hormone. Since 1981, a group of active related peptides has been isolated, purified, peptide sequences determined, and synthesised (Buckalew et al., 1987; Ballerman, 1987). The polypeptide precursor molecule has been identified by cDNA sequencing as a single 151 amino-acid molecule in man, termed pre-pro-atrial natriuretic peptide, though the principle storage form, pro-ANP, consists of 126-amino acids. A 28-amino acid residue has been isolated from human atrial tissue, and the recent development of sensitive radioimmunoassay confirms that this moiety, named α -hANP, is the normal circulating form in man, with normal plasma levels in the region of 10-70 pg/ml (Needleman and Greenwald, 1986; Buckalew et al., 1987). Dietz (1984) demonstrated in the rat heart-lung preparation that atrial distension produced a marked rise in ANP release. ANP release and increased plasma ANP levels have been demonstrated in various experimental manoeuvres in animals and man, including intravenous volume expansion (Yamaji et al., 1985), headout water immersion (Ogihara et al., 1985), and chronic elevation of dietary salt intake (Shenker et al., 1985), and increased central venous and hence atrial pressure may be the physiological stimulus for hormone release. Very high levels have been identified in pathological conditions associated with circulatory overload, congestive cardiac failure (Shenker et al., 1985) and chronic renal failure (Espiner et al., 1985), and in tachyarrhythmias (Tikkanen et al., 1985). Infusion of angiotensin II, vasopressin and pheynylephrine also increase ANP release (Manning et al., 1985). Such stimulation has been shown for vasopressin and α adrenergic agonists in vitro (Sonnenberg and Veress, 1984) suggesting that the response is not purely due to pressor effects.

Experimental models, which have included infusion into whole animals

and man, and isolated tissue and cellular preparations, have shown ANP to have a number of important actions in the body. It seems clear that ANP mediates its effects through the intracellular second messenger, cGMP (Hamet et al., 1986). Specific high affinity receptors have been identified in vascular, adrenal and renal tissue and in the brain (Ballerman, 1987). ANP relaxes pre-constricted blood vessels in vitro (Kleinert et al., 1984) and reduces blood pressure in vivo (Weidmann et al., 1986). Suppression of plasma renin activity, basal and angiotensin II stimulated aldosterone release, and vasopressin release, are known hormonal effects (Samson, 1985; Anderson et al., 1986). There is debate about the mechanism of its effects in the kidney. Increased glomerular filtration rate and glomerulotubular imbalance (Huang et al., 1985), redistribution of renal blood flow to deeper salt-losing nephrons (Cole and Needleman, 1985) and direct tubular effects have all been postulated to account for its marked natriuretic response. In the kidney, ANP receptors and cGMP production have been demonstrated in the glomeruli and papillae, and isolated tubular and cell studies suggest that sodium reabsorption is inhibited in the cortical and medullary collecting duct (Sonnenberg et al., 1982; Zeidel et al., 1987). The proximal tubule is devoid of ANP receptors and cGMP activity in response to ANP (Genest, 1986), and evidence for a proximal site of action from lithium and free water clearance techniques probably reflects an indirect effect such as inhibition of angiotensin II formation (Buckalew et al., 1987).

Most of the reported studies have utilised ANP at a dose that represents a truly pharmacological stimulus, and it is not yet possible to accord to ANP its correct physiological status. Specific inhibitors available for use in man will clarify the picture when available and in this context, Naruse et al., (1985) have demonstrated reduced urinary sodium excretion after inoculation of specific ANP-antiserum into rats. Some groups have attempted to infuse ANP at

such small doses that blood levels produced are at least comparable to the elevated levels found in the pathological conditions mentioned. Such studies in man have demonstrated a smaller but significant natriuresis, with renin and aldosterone suppression, but without change in blood pressure (Cuneo et al., 1986; Anderson et al., 1987), and natriuresis without alteration of glomerular filtration rate was shown in low dose studies in animals (Yukimura et al., 1984)

The discovery of ANP holds the promise of a novel mechanism for body sodium homeostasis. The mutual regulatory interaction of vasopressin, angiotensin II and the catecholamines with ANP, provides the basis for integrating afferent mechanisms that respond to central blood volume, with variation in systemic blood pressure and the capacity to excrete salt.

The Kallikrein-kinin system

In addition to its significance in the systemic circulation, all components of the kallikrein-kinin system are present within the kidney. Much research has attempted to define a role for the system in the kidney but as yet it remains of potential but unproven significance. Renal kallikrein, a serine protease, is synthesised in the distal tubule, and acts on filtered low molecular weight kininogen to produce lysylbradykinin (kallidin) which is biologically active. In man, bradykinin is probably formed enzymatically in the urine. The kinins are broken down by kininases present in proximal and distal tubules (Scicli and Carretero, 1986).

The activity of the renal kallikrein-kinin system is usually inferred from measuring urinary kallikrein excretion. However, the relationship to kinin release is unclear and Vinci et al. (1977) were unable to demonstrate a correlation between the two components in urine.

Intra-arterial renal injection of kinins results in vasodilatation and

natriuresis (Carretero and Scicli, 1980) and from such studies a physiological role has been ascribed to the system. However, the presence of kininases in high concentration at the proximal tubule results in complete breakdown of kinins administered in this way, with minute amounts appearing at the distal tubule, the site of endogenous production (Nasjletti et al., 1975). In addition, it is unclear whether renal kallikrein releases kinins into the renal circulation to exert vasoactive effects. It is not yet clear whether kinins directly affect tubular sodium handling, but those studies suggestive of an effect, point to a distal site, particularly the collecting duct (Jackson et al., 1985).

The kinin-kallikrein system appears to have a reciprocal stimulatory relationship with the prostaglandins. Thus kinins stimulate phospholipase A_2 , releasing arachidonic acid, the precursor for a range of prostaglandins. Conversely, phospholipase A_2 can activate membrane bound renal kallikrein, and prostaglandins increase urinary kallikrein excretion (Scicli and Carretero, 1986). Studies with cyclooxygenase inhibition suggest that the prostaglandins may mediate, at least in part, the renal effects of infused kinins (Cinotti, 1983), and prostaglandins are involved in the antagonism of vasopressin by kinins (Schuster et al., 1984b).

The kallikrein-kinin system interacts with the renin-angiotensin-aldosterone axis at a number of points. Urinary kallikrein activates pro-renin in vitro (Sealey et al., 1978), and in vivo, bradykinin infusion stimulates renin release (Flamenbaum et al., 1979). Aldosterone is a potent stimulus to renal kallikrein synthesis (Marin-Grez, 1982). In addition, Kininase II is identical to angiotensin converting enzyme. Kinins are elevated by angiotensin converting enzyme inhibitors and may contribute to drug effects. A low sodium diet produces a rise in kallikrein excretion (Margolius et al., 1974). Such a finding and the known hormonal interactions have led some observers to postulate that the

prostaglandins and kinins act synergistically to counteract the antinatriuretic, renal vasoconstrictor action of the renin-angiotensin-aldosterone axis under conditions of salt depletion.

Whether the kallikrein-kinin system contributes to natriuresis under conditions of sodium excess is unclear. As discussed, the system appears to be principally activated in salt depletion. Intravenous salt loading has been reported to increase urinary kallikrein excretion (Mills et al., 1976). However, many stimuli that increase urine flow rate including diuretics, water loading and saline loading, increase kallikrein excretion in a way that may be artefactual and represent a "wash-out" phenomenon (Marin-Grez, 1982). In spite of this, the administration of antibodies against kinins to saline-expanded rats has been reported to result in a decrease in sodium excretion (Marin-Grez, 1974), so the situation is not yet resolved.

Other hormonal influences on sodium excretion

In addition to the above factors, experimental evidence has been presented for a distinct natriuretic hormone involved in renal salt-handling. Cross circulation experiments in animals have indicated the existence of a transferrable hormonal factor in three separate models: mineralocorticoid escape, progressive nephron loss and body fluid volume expansion. The nature of the factor(s) has not yet been elucidated. There is however, evidence that it derives from the hypothalamus, is a normal constituent of plasma, and inhibits sodium transport at the nephron. As distinct from established hormones producing effects at the kidney, its cellular mode of action involves inhibition of Na^+/K^+ -ATPase (reviewed by Sonnenberg, 1986). In addition to a possible physiological role, it has been implicated in the pathogenesis of essential hypertension, in which a compensatory increase in activity serves to augment

sodium excretion by the kidney (de Wardener and MacGregor, 1982).

Heller (1987) has reviewed the evidence for a number of other substances which may regulate intrarenal haemodynamics, under physiological and pathophysiological conditions. The potential renal importance of these mediators, which include serotonin, neuropeptide Y, adenosine and histamine, is becoming an area of active interest.

2) DOPAMINE AS AN ENDOGENOUS RENAL HORMONE

In this section evidence for the renal synthesis of dopamine will be reviewed. The distinct actions of the amine at the kidney and on other renally associated hormones will be discussed with an attempt to place dopamine into the framework of the mechanisms of natriuresis already referred to.

Dopamine is the natural precursor for noradrenaline and adrenaline and the metabolic pathway for catecholamine synthesis is depicted in Figure 1. The relevant enzymes are present in adrenergic neurones and the adrenal medulla, in which the formation of L-dopa from L-tyrosine, under the action of tyrosine hydroxylase, is the rate limiting step.

An independent physiological role for dopamine became accepted when evidence accrued that it acted as a neurotransmitter in its own right in the central nervous system. Dopamine is the principle catecholamine within the basal ganglia, and three principle dopaminergic systems have been identified - the nigrostriatal, mesolimbic and tubero-infundibular pathways. Further details concerning dopamine in the central nervous systems are beyond scope of this review.

That dopamine might have a distinct role in the periphery, and particularly at the kidney, separate from that purely as a precursor, was suggested by two separate lines of evidence. First was the demonstration that it is present in high concentration in renal tissue and urine, relative to noradrenaline and adrenaline. Secondly, infused dopamine was shown to possess a unique profile of cardiovascular and renal actions, leading to the demonstration of specific dopamine receptors.

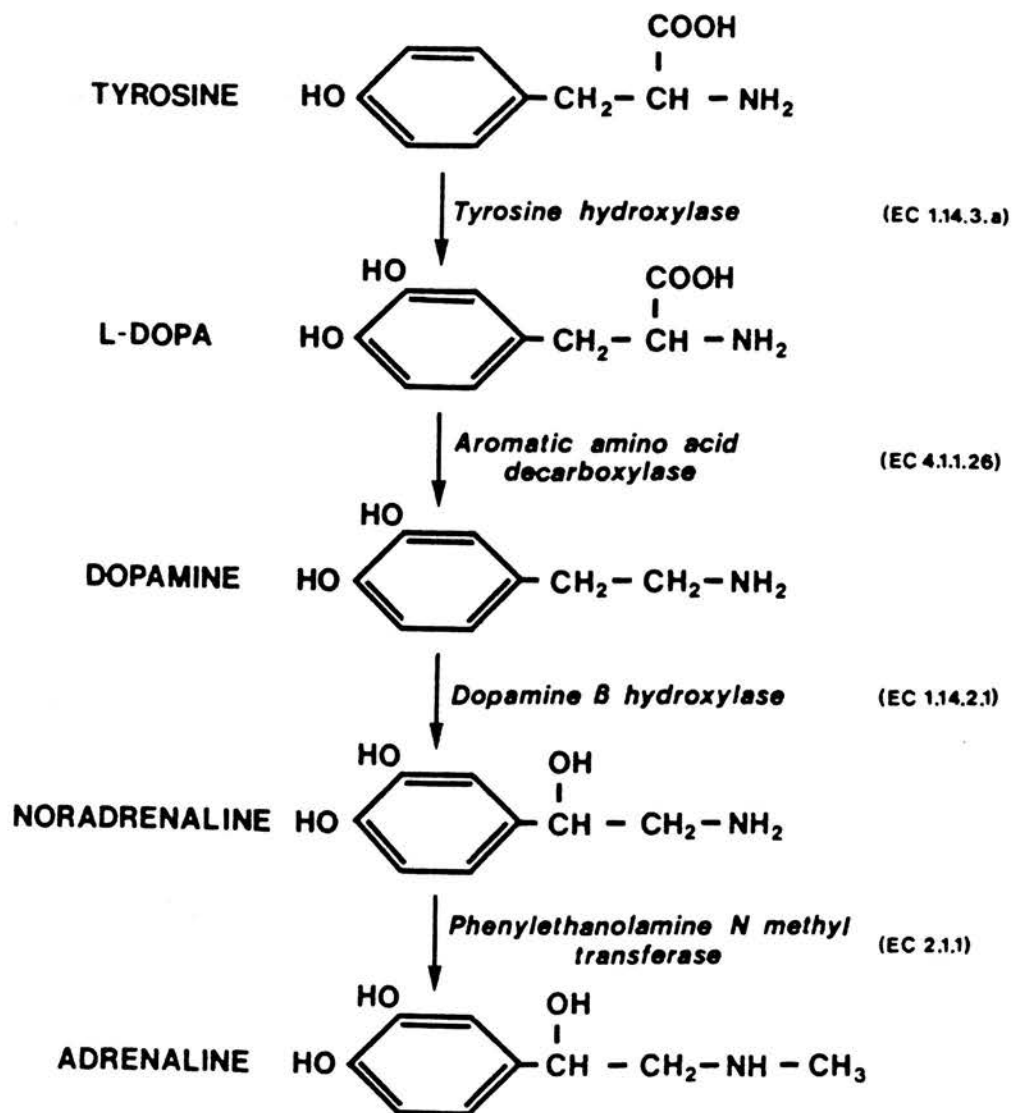


FIG. 1. The biosynthetic pathway for the catecholamines

Renal synthesis and urinary excretion of dopamine

Free dopamine is found in large amounts in the urine of man, and is the major excreted catecholamine (Sourkes and Drujan, 1957). Reported values for free catecholamines in man are: adrenaline 0-82 nmol/24h, noradrenaline 118-414 nmol/24h and dopamine 653-2285 nmol/24hr (Crout, 1968). A similar pattern pertains to the rat, but not to the dog, where noradrenaline predominates (Ball et al., 1982).

There has been some debate concerning the source of urine dopamine and it was previously believed to derive from circulating plasma dopamine. With the development of sensitive radioenzymatic methods for catecholamine estimation, various groups have reported plasma free dopamine levels, values ranging between 40 and 330 pg/ml (reviewed by Van Loon and Sole, 1980). Ball et al. (1978a) measured dopamine in plasma and urine from six subjects and demonstrated a mean urinary clearance of 1996 ml/min. Although dopamine can undergo renal tubular transport (Rennick, 1968), it was evident that even with filtration and secretion, plasma dopamine could not quantitatively account for normal levels of free dopamine in urine. The obvious conclusion was that the kidney must actively synthesise dopamine.

To account for the levels of dopamine in urine, three hypothesis have been forwarded:-

1. Deconjugation in the kidney of circulating plasma dopamine conjugates.
2. Renal dopaminergic nerves.
3. Renal decarboxylation of circulating plasma L-dopa.

Dopamine conjugates, principally the sulphate and glucuronide, form the major portion (> 95%) of total plasma dopamine, with values in the nanogram range. In addition, levels in urine are ten times those of the free amine

(Kuchel et al., 1979). The conjugated forms are traditionally believed to represent inactive, metabolic end-products of the free amine (Anton, 1987). Kuchel's group have however, suggested that dopamine sulphate can be converted to noradrenaline (Buu and Kuchel, 1979), or deconjugated to free dopamine in the kidney (Unger et al., 1978), thereby effecting a transport or storage function for the free amine. There is however, little confirmatory evidence. Bradley et al. (1985) infused dopamine-4-sulphate into the renal artery of the dog. The preparation was inactive in terms of renal haemodynamic effects, and no conversion to free catecholamines in urine could be demonstrated. Similarly, in the isolated, perfused rat kidney preparation, the addition of dopamine-4-sulphate to the perfusate did not result in an increase in urine free dopamine (Adam and Adams, 1985). The most compelling evidence against dopamine conjugates contributing to free urine dopamine comes from the work with the dopa decarboxylase inhibitor, carbidopa. In studies in man (Williams et al., 1986) and in the isolated perfused rat kidney (Baines and Drangova, 1984; Adam and Adams, 1985), carbidopa can be shown to markedly suppress or abolish urine dopamine excretion, strongly suggesting that the action of dopa decarboxylase is a prerequisite for free dopamine synthesis and release into the urine.

The first evidence for the existence of functional dopaminergic nerves in the kidney came in 1973. Electrode stimulation of the mid-brain in the anaesthetised dog produced a renal vasodilator response which could be blocked by the dopamine antagonist, haloperidol (Bell and Lang, 1973). Dinerstein et al. (1979) followed up this work by demonstrating, using histofluorescence techniques in the canine kidney, a population of neuronal elements at the glomerular vascular pole which contained predominantly dopamine. Further evidence came from a study by Chapman et al. (1979). In rats, renal vasodilat-

ion was produced after renal nerve stimulation in the presence of α -adrenergic blockade. That vasodilation was due to a dopaminergic response, was suggested by blockade of the effect with sulpiride. However, Bradley et al. (1986a) were unable to demonstrate any effect of dopamine receptor blockade on the renal response to renal nerve stimulation, and have drawn attention to the fact that numerous studies have failed to confirm the anatomical basis and functional role for dopaminergic nerves in the kidney. There is no doubt that manoeuvres which activate the renal nerves, either renal nerve stimulation (Bradley and Hjendahl, 1984), or bilateral carotid occlusion (Bradley et al., 1987), lead to the release of both noradrenaline and dopamine. It is unclear from such observations whether dopamine is present within noradrenergic neurones, the two amines being released concurrently on renal nerve stimulation, or whether dopamine is being released from a specific and distinct population of neurones. It is as yet uncertain to what degree dopamine released from neuronal tissue, either noradrenergic or dopaminergic, contributes to free dopamine levels in urine. In animals, chronic denervation produces either no change (Stephenson et al., 1982; Baines and Drangova, 1984; Adam and Adams, 1985) or only a modest reduction in urine dopamine (Morgunov and Baines, 1981; Baines, 1982). Christensen et al. (1976) studied tetraplegic patients with physiologically complete cervical spinal cord transection above the sympathetic outflow. Urinary excretion of dopamine was not significantly different from normal values whereas urinary noradrenaline and adrenaline were markedly reduced as would be expected. In another study in man (Williams et al., 1986), carbidopa reduced urinary dopamine excretion without affecting urinary noradrenaline. The authors suggested that intraneuronal blockade of dopa decarboxylase would have been expected to lower noradrenaline as well as dopamine, and postulated that dopamine in urine is produced by extraneuronal decarboxylation of L-dopa.

The isolated perfused rat kidney actively synthesises dopamine in the absence of renal innervation, and the addition of L-dopa to the perfusion medium results in a large increase in dopamine into the urine, a process blocked by carbidopa (Suzuki et al., 1984; Adam and Adams, 1985). When the L-dopa concentration in the perfusate was in the range of the physiological plasma concentration, urine dopamine excretion was comparable to that observed in normal rats. However, a dopamine level comparable to the *in vivo* state produced very little dopamine in urine (Suzuki et al., 1984). Similarly, in rats studied *in vivo*, the administration of L-dopa produced a large increase in urinary dopamine excretion (Sato et al., 1987). Ball et al. (1982) measured the arteriovenous difference for dopamine and L-dopa across the kidney in anaesthetised dogs. Whereas dopamine was added to the urinary and venous outflow, L-dopa was extracted by the kidney. A similar situation has been demonstrated in man. An oral load of L-dopa (250 mg) produced similar maximal increments in plasma L-dopa and urine dopamine (98- and 93-fold respectively). There was a high correlation in the subjects between plasma L-dopa concentration and urine dopamine output in both the baseline state and after the administration of L-dopa (Brown and Dollery, 1981). In six subjects undergoing catheterisation to exclude pheochromocytoma, the same group measured the arteriovenous difference across the kidney for L-dopa and dopamine (Brown and Allison, 1981). They confirmed that L-dopa is extracted to a much higher degree than accountable for by its urinary excretion, and estimated that circulating L-dopa could produce up to 75% of the measured level of dopamine in urine.

The kidney is known to contain a high concentration of the enzyme dopa decarboxylase, which is concentrated at the proximal tubule (Goldstein et al., 1972). In histofluorescence studies, Hagege and Richet (1985), elegantly demon-

strated dopamine synthesis in proximal tubular cells of rat kidney cortex slices, a process stimulated by L-dopa, and blocked by the dopa decarboxylase inhibitor, benserazide. In a micropuncture study in rats, Baines and Chan (1980) injected L-dopa into the proximal tubule and demonstrated active dopamine synthesis and release into the urine. There was no difference in response when innervated and chronically denervated kidneys were compared.

In summary, although data have been derived from widely differing experimental models, and results are at times conflicting, a reasonable synthesis of reported work would be that under normal circumstances, free dopamine, excreted into urine, derives from the extraneuronal decarboxylation of circulating L-dopa in proximal tubular cells. The putative dopaminergic nerves require further delineation in functional and anatomical terms, but neuronal sources do not appear to make an important contribution to levels of dopamine in the urine.

Dopamine receptors

The identification and characterisation of dopamine receptors has attracted great interest but also much controversy, and the subject has been widely reviewed (Lokhandwala and Barrett, 1982; Keabian and Calne, 1979; Goldberg and Kohli, 1983). In 1979, Keabian and Calne proposed the existence of two dopamine receptors in the central nervous system, which they designated D_1 and D_2 . Characterisation depended on the production of a biochemical response, namely stimulation of adenylyl cyclase. Activation of the D_1 receptor (eg. in the caudate nucleus) is associated with stimulation of adenylyl cyclase. Activation of D_2 receptors (eg. in the striatum or anterior pituitary) either

produces no effect or leads to inhibition of cAMP release in brain tissue. Further studies on dopamine receptors in the central nervous system have been performed using radioactive labelled ligand binding assays. Conflicting results have been presented and the existence of up to four distinct types of dopamine receptors (or binding recognition sites), postulated to account for findings (Stoof and Kebabian, 1984).

There is still no definite evidence that dopamine functions as a neurotransmitter in the periphery. However, studies with infused dopamine and agonists have defined specific receptors which can be pharmacologically manipulated. The basis for characterising peripheral dopamine receptors has classically been by producing agonist and antagonist potency profiles with regard to a physiological response. Goldberg and Kohli (1983) produced a classification which divided peripheral receptors into DA_1 and DA_2 subtypes. It must be noted that this classification has been derived by different criteria to that for receptors in the central nervous system and direct comparisons are not straight forward. The DA_1 receptor is situated on vascular smooth muscle and is known as the musculotropic or post-synaptic receptor. A number of models have been developed for analysis of the DA_1 receptor, both in vivo (eg. the dog mesenteric and renal vascular beds) where stimulation results in vasodilation, and in vitro (eg. the rabbit isolated splenic artery) where vascular relaxation is the functional response (Brodde, 1982; Shepperson et al., 1982). There is now substantial evidence that DA_1 receptors are present on coronary, cerebral, hepatic and femoral, as well as renal and mesenteric vascular beds (Lokhandwala and Barrett, 1982). The DA_2 receptor is situated pre-junctionally on sympathetic nerve terminals, and it is also known as the presynaptic neuronal or neurotropic receptor. Activation results in inhibition of stimulus-evoked noradrenaline release. Again, both in vivo and in vitro models have been utilised to

characterise the DA₂ receptor (reviewed by Hilditch and Drew, 1987). Lokhandwala and Buckley (1977) measured the effect of dopamine on vasoconstrictor responses to sympathetic nerve stimulation and provided evidence for DA₂ receptors on renal sympathetic nerves.

Dopamine receptors have also been identified on sympathetic ganglia, activation of which results in hyperpolarisation and inhibition of ganglionic transmission. The receptor type has not yet been fully determined (Lokhandwala and Barrett, 1982). Evidence for the presence and subtype of dopamine receptors on cells of the renal tubule, juxtaglomerular apparatus and adrenal cortex will be discussed in the next section. Dopamine itself has activity on both DA₁ and DA₂ receptors and in addition stimulates α and β adrenergic receptors (Goldberg, 1972). The models developed have allowed analysis of the structural and stereospecific requirements for agonists at the dopamine receptors. From such work, a number of compounds have been synthesised which have relative specificity at each receptor, at the same time possessing little affinity for other receptors, and potency series have been defined.

The benzazepine derivative, fenoldopam, appears to be the most specific agonist at the DA₁ receptor, whereas the partial ergoline, LY 171555 is a selective DA₂ agonist (Harvey et al., 1986; Caverio et al., 1987).

The first dopamine receptor antagonists, active in the central nervous system, were the major tranquillisers of the phenothiazine, butyrophenone and thioxanthine groups. They also have action on peripheral receptors, but most of the compounds antagonise both DA₁ and DA₂ receptors. More recently, a number of specific compounds have been developed which have greatly facilitated study of receptor mechanisms. Thus domperidone and the benzazepine, SCH 23390, are highly selective antagonists at DA₂ and DA₁ receptors, respectively (Glock et al., 1982; Goldberg et al., 1984). The (+) enantiomer of

sulpiride is the most selective DA_1 antagonist available for use in man, whereas the (-) enantiomer is more than 100 times more active than the (+) form at the DA_2 receptor (Goldberg and Kohli, 1983).

The development of highly selective agonists and antagonists has allowed comparisons to be made between central and peripheral dopamine receptors in terms of potency series. In addition, binding assays and cyclic AMP responses have been determined in various models of peripheral receptors (Brodde, 1982). Goldberg and Kohli (1981) have reviewed the evidence for similarities between the receptor systems and also pointed to the discrepancies that remain. The latter may reflect differences in experimental design or indicate that the central and peripheral receptors are chemically distinct.

The functional significance of dopamine receptors on the systemic circulation is unclear. In view of the very low plasma concentration, it seems unlikely that circulating dopamine influences vascular receptors under normal conditions. In addition, receptor antagonists have not been reported to increase systemic resistance or blood pressure which might suggest that dopamine is exerting a relaxant influence on the vasculature. The mechanism by which endogenous dopamine activates neurotropic receptors is unknown. Mannelli and Pupilli (1988) have reviewed evidence for the concurrent release of dopamine and noradrenaline from sympathetic nerves, and suggest that the quantity of dopamine released may be locally sufficient to activate DA_2 receptors. However, in most models tested, receptor antagonists do not enhance noradrenaline release, and a physiological role for the peripheral DA_2 receptor cannot yet be defined (Berkowitz, 1983; Mannelli and Pupilli, 1988). Evidence for the role of dopamine receptors in the kidney will be reviewed in the next section.

Pharmacological and physiological effects of dopamine

When McDonald and co-workers (1964) infused dopamine into man, it was evident that it was exerting unique effects in the circulation, with increased renal blood flow and natriuresis, and a rise in cardiac index without change in blood pressure or pulse. Such actions were distinct from those produced by other catecholamines. Meyer et al. (1967) demonstrated that the augmentation of renal function was not secondary to systemic haemodynamic changes, as administration of dopamine into one renal artery produced marked ipsilateral effects with little change at the other kidney. Renal vasodilatation has now been demonstrated in man and animals by various techniques including electromagnetic flow meter, clearance of para-aminohippuric acid (PAH), indicator dye dilution and renal angiography (reviewed by Goldberg, 1972). The optimal dose in man is around 5 $\mu\text{g}/\text{kg}/\text{min}$ (D'Orio et al., 1984). Above this dose, α and β adrenergic receptors are increasingly stimulated and most observers utilise blocking agents on these receptors to characterise the dopamine response (Goldberg, 1972, Goldberg and Weder, 1980). In the rat, dopamine normally produces vasoconstriction at all doses, and α -blockade is required to unmask specific dopaminergic actions (Chapman et al., 1980). The concept that dopamine causes renal vasodilation by action on a specific receptor was strengthened by the discovery of specific dopamine blocking agents. Thus renal vasodilatation was attenuated by haloperidol (Yeh et al., 1969), chlorpromazine (Brotzu, 1970) and sulpiride (Chapman et al., 1980), but not by adrenergic, cholinergic or antihistamine antagonists (Goldberg, 1972).

The action on GFR is not consistent. Although the study of McDonald et al. (1964) showed a 25% increase, many studies have not demonstrated significant rises in GFR (Levinson et al., 1985; Frederickson et al., 1985). This lack of

consistent effect may be explained in part by the study of Edwards (1985) who indicated that dopamine relaxes both pre- and post-glomerular arterioles, so that glomerular hydrostatic pressure may remain unaltered.

There has been much interest in the mechanism of natriuresis following dopamine. Haemodynamic changes alone could partly account for the effect. A reduction in filtration fraction, by altering peritubular physical forces, will result in reduced proximal tubular sodium reabsorption. In addition, Hardaker and Wechsler (1973) demonstrated in dogs that dopamine causes a redistribution of blood flow from outer to inner cortex, and reduction in extraction of PAH suggests shunting from cortical to medullary areas (Goldberg, 1972). Such change may result in relative 'washout' of the medullary interstitium and reduced sodium reabsorption at the ascending limb of the loop of Henlé. However, various workers have indicated, both with dopamine and agonists, that natriuresis can occur in the absence of renal haemodynamic changes (McGiff and Burns, 1967; Carey and Hughes, 1987), and direct tubular actions have been invoked. In an early micropuncture study, Seely and Dirks (1967) pointed to a proximal tubular site of action, though around the same time, Davis et al. (1968) provided evidence for a distal effect without alteration in GFR. More recently, the question has been addressed by in vitro tubular preparation techniques. Dopamine receptors have been identified in the proximal tubule of the rabbit by [3 H]haloperidol binding (Felder et al., 1984). Bello-Reus et al. (1982) have shown that dopamine inhibits fluid reabsorption across rabbit proximal tubules. The cellular mechanism of action at the proximal tubule is not yet clear, but in rat proximal tubule segments, dopamine has been shown to inhibit Na^+/K^+ -ATPase (Aperia et al., 1987). It appears therefore, that the natriuresis consequent upon dopamine administration has both a haemodynamic and a direct tubular component.

Most studies on the dopamine renal vascular receptor have focused on models in which physiological measurements of changes in blood flow or vascular relaxation have been made. In the brain, receptors have been characterised by determination of a biochemical response, namely adenylyl cyclase activity and radioactive ligand binding. More recently, groups have attempted to determine cAMP responses in the kidney after stimulation by dopamine and dopamine agonists. Nakajima and colleagues (1977) defined a dopamine-sensitive adenylyl cyclase in a rat kidney particulate preparation, and dopamine stimulates cAMP release in the canine renal artery (Murthy et al., 1976; Sabouni et al., 1987). Kotake et al. (1981) identified a dopamine-sensitive adenylyl cyclase in rat glomeruli and found a similar order of potency in ability to stimulate cAMP, in a series of dopamine agonists, to that in striatal homogenates. Schwartz's group (1982) used the dog isolated kidney and found a high correlation in a group of dopaminomimetics, between renal vasodilation and stimulation of dopamine-sensitive adenylyl cyclase. The studies described suggest firstly that central D_1 and renal DA_1 receptors may be similar, and secondly that vasodilation of the renal vasculature is mediated through DA_1 receptors. In the study reported by Felder et al. (1984), both dopamine and the DA_1 agonist fenoldopam stimulated cAMP release in the proximal tubule. Thus both the renal vasodilator and natriuretic capacity of dopamine appears to be dependant on DA_1 receptor activation. This is confirmed in dogs in which the renal effects of dopamine were abolished by pretreatment with SCH 23390, the most specific DA_1 receptor antagonist available, whereas the DA_2 antagonist domperidone was without significant effect (Frederickson et al., 1985). Whether cAMP mediates the action of dopamine after DA_1 receptor stimulation is unknown. However in dogs, infusion of dibutyryl cAMP produced a natriuresis (Gill et al., 1971) and in rabbit kidney brush border membranes cAMP inhibited the Na^+/H^+ antiporter

(Weinman et al., 1987).

The kidney is abundantly supplied with DA₂ receptor-containing adrenergic nerves. Most studies in animals, as indicated, involve the use of α and β blockers to characterise dopaminergic responses, which would be expected to prevent the expression of DA₂ receptor-mediated inhibition of noradrenaline release from the renal sympathetic nerves. Thus Seri et al. (1987) showed in the rat that the DA₂ agonist lisuride reduced renal vascular resistance, and in the presence of the DA₂ antagonist S-sulpiride, dopamine did not significantly influence renal blood flow.

Although from the evidence reviewed, dopamine exerts potent pharmacological effects on renal haemodynamics and salt excretion, by activating specific receptors, less work has been directed towards identifying physiological actions for the catecholamine in the kidney. Some studies involving the use of dopamine receptor antagonists or inhibitors of dopamine synthesis suggest that it may play a role in normal renal function. In a study already referred to (Chapman et al., 1980), sulpiride produced vasoconstriction in the rat renal cortex as assessed by the H₂ washout technique. The authors suggested that endogenous dopamine normally regulates cortical blood flow by maintaining vasodilatation. In two studies in man, dopa decarboxylase inhibition with carbidopa was demonstrated to inhibit dopamine excretion, with both acute, and chronic dosing over 3 days. In both cases, sodium output was significantly reduced, compared to controls (Williams et al., 1986; Ball and Lee, 1977a), implying that under normal conditions dopamine has a tonic influence on renal salt handling. Another approach has been to determine the natriuretic response to volume expansion consequent upon acute intravenous salt loading. In rats undergoing saline-induced diuresis, both haloperidol, an unselective dopamine antagonist, and the DA₁ receptor antagonist SCH 23390, reduced the natriuret-

ic response by 25.2% and 30.3% respectively (Schnermann et al., 1986). In dogs, carbidopa attenuated maximal salt excretion by around 40% after intravenous saline (McClanahan et al., 1985).

It must be appreciated however, that not all studies demonstrate an effect of dopamine in the physiological state and the subject will be discussed in greater detail in the light of results to be presented.

The effect of dopamine on renin release from the juxtaglomerular cells has been studied in animals and man, both in vivo and in vitro. In rat kidney slices and the isolated rat kidney, dopamine stimulated renin release. The effect was blocked by propranolol, leading both groups (Henry et al., 1977; Quesada et al., 1979) to suggest that the stimulation was β -receptor mediated. However, Williams et al. (1983) showed, using isolated renal cortical cells, that sulpiride attenuated the renin response to dopamine, suggesting the presence of specific dopamine receptors. Ayers et al. (1969) and Otsuka et al. (1970) infused dopamine into dogs and showed stimulation of renin at high dose levels (12 $\mu\text{g/kg/min}$), again suggesting that the response was mediated through the β receptor. However, Imbs et al. (1975) and Dzau et al. (1978) used lower doses (6 $\mu\text{g/kg/min}$ and 0.28 to 3.0 $\mu\text{g/kg/min}$ respectively) and produced increased renin release. Under these circumstances the increase was prevented by the dopamine antagonist haloperidol, but not by propranolol. More recently, the renin response to low dose intrarenal dopamine infusion in conscious dogs was inhibited by sulpiride, but not by propranolol (Mizoguchi et al., 1983).

Results have similarly been inconclusive in man. Wilcox et al. (1974) showed that dopamine, in doses causing a pressor response, elevated plasma renin activity. However, Levinson et al. (1985) infusing dopamine at between 0.03 and 3 $\mu\text{g/kg/min}$ did not report any change in renin activity.

The known influences on renin release have been reviewed. Dopamine

can interact with these in a number of ways. It may directly activate β and putative dopaminergic receptors on the juxtaglomerular cells, and may inhibit sympathetic nerve activity by its DA_2 effects. In addition it affects both the baroreceptor and macula densa mechanisms by producing renal vasodilation and natriuresis. The overall effect must therefore represent a balance of competing influences and in the studies performed, the varying dosage, species, state of anaesthesia, and systemic effects of blood pressure changes may account for the discrepancies in the results.

In addition, it must be questioned whether circulating dopamine forms the physiological moiety for controlling renin release. L-dopa, as the precursor for dopamine, has been shown actually to inhibit plasma renin activity (Worth, 1986) and the question will be discussed in some detail in the light of the results to be presented with the renal dopamine prodrug, γ -L-glutamyl-L-dopa.

In addition to the well described stimuli to the secretion of aldosterone, namely potassium, adrenocorticotrophic hormone and angiotensin II, studies in whole animals and man, and in vitro, have suggested that dopamine provides a further inhibitory input to the adrenal cortex (reviewed by Ganguly, 1984; Sowers 1984; Fraser, 1987). Neither the infusion of dopamine, nor the administration of bromocriptine affects aldosterone in the baseline state (Carey et al., 1980; Noth et al., 1980). However, the dopamine antagonist metoclopramide actively stimulates aldosterone release (Norbiato et al., 1977) and dopamine inhibits this response in a dose dependant manner (Carey et al., 1980; Noth et al., 1980). In addition, dopamine inhibits the angiotensin II-induced rise in aldosterone in man during sodium restriction (Drake et al., 1984). These findings have led to the hypothesis that in sodium replete subjects, aldosterone is under maximal tonic dopaminergic inhibition, which can be blunted either by metoclopramide or by salt restriction.

In vitro, McKenna et al. (1979) reported that dopamine inhibits aldosterone biosynthesis induced by angiotensin II. Dopamine levels achieved during in vitro studies are extremely high and it is relevant to the interpretation of such studies, that, in rat zona glomerulosa tissue, dopamine occurs at concentrations up to 10,000 times plasma levels (Inglis et al., 1987). The source for such large concentrations of dopamine is unknown. There is at present no evidence for dopaminergic innervation to the adrenal cortex. Whether the uptake and local decarboxylation of circulating L-dopa, in an analagous manner to events in the kidney, provides the source of dopamine, awaits further study.

Bevilacqua et al. (1982) presented evidence for DA₁ receptors on calf adrenal cells. Stern et al. (1986) used a bovine zona glomerulosa cell model and ³H-spiperone ligand binding to characterise dopamine receptors. The greater potency of the DA₂ agonist LY 171555 relative to the DA₁ agonist fenoldopam, and lack of cAMP response to dopamine points to the importance of the DA₂ receptor in mediating the effects of dopamine. However, many questions remain. Most studies on antagonists have been performed with the relatively non-specific agent, metoclopramide, and it is of note that the specific DA₂ antagonist, domperidone, does not influence aldosterone release (Sowers et al., 1982).

During conditions of high sodium intake, inhibition of the aldosterone response to angiotensin II would complement the renal vascular and direct tubular action of dopamine, in promoting natriuresis. Although such a synthesis is attractive, the action of dopamine on the adrenal cortex under physiological conditions is not yet fully defined.

A number of groups have shown that the infusion of dopamine into the dog and man does not alter plasma vasopressin levels, arguing against a role for circulating dopamine in the control of vasopressin release or destruction

(Ball et al., 1981; Morton et al., 1985; Os et al., 1987a). In an early study, Deis and Alonso (1970) infused dopamine into the rat and postulated that the diuresis produced was due to an inhibition of the action of vasopressin. However, neither sodium output nor vasopressin levels were measured. In dogs, dopamine produced a diuresis and reduction in urine osmolality but the renal effects of concurrently infused vasopressin were unaltered by dopamine (Cadnapaphornchai et al., 1977). More recently, Koyama et al. (1985) have suggested in the rat that dopamine exerts a dose-dependant inhibitory effect on the renal action of vasopressin. As exogenous dopamine does not penetrate the blood-brain barrier (Bertler et al., 1966), a central effect of locally synthesised dopamine on vasopressin release is a possibility. Studies in man utilising centrally acting dopamine antagonists have not produced a consensus. Fluphenazine and chlorpromazine have been reported to increase vasopressin levels in plasma (De Rivera, 1975; Shah et al., 1973) but metoclopramide and haloperidol were without effect (Os et al., 1987b; Kendler et al., 1978). Lightman and Forsling (1980) demonstrated, in man, that infused L-dopa suppressed resting levels of vasopressin and inhibited the rise produced by head-up tilt. Interestingly, the response was blocked by carbidopa and they suggested that inhibition of vasopressin must occur at a site outwith the blood brain barrier, either at the pituitary level or at the median eminence of the hypothalamus. This was partially confirmed by Reid et al. (1986) who reported a marked inhibition of vasopressin after L-dopa administration to dogs, but in this model carbidopa did not prevent the inhibition. Various groups have attempted to study dopaminergic control of vasopressin by direct injection of dopamine into animal brains, but results have been contradictory (reviewed by Forsling and Williams, 1984).

Cuche et al. (1976) have suggested that dopamine can influence the

renal clearance of phosphate. Under conditions in which parathyroid hormone, and other catecholamines were controlled, the infusion of dopamine or L-dopa into the renal artery of the dog resulted in an increase in urinary phosphate excretion, a response that was dissociated from the increase in renal blood flow and natriuresis. The physiological importance of this observation is unclear as inhibition of dopamine synthesis by carbidopa was without effect under basal conditions. Kaneda and Bello-Reus (1983) have demonstrated in isolated rabbit nephron segments, that dopamine inhibited the flux of phosphate across the proximal tubule.

The effect of dopamine on other hormones in the kidney, and renal hormonal interactions between dopamine and the prostaglandins and kinin-kallikrein system, and atrial natriuretic peptide, have been studied by a number of workers without a consensus emerging. These topics will be discussed in greater detail in relation to results presented in the present studies.

Factors influencing the renal synthesis and urinary excretion of dopamine

Alexander and colleagues (1974) first demonstrated that dietary manipulation could alter the urinary output of free dopamine in man. Increasing sodium intake from 9 to above 200 mmol per day resulted in a 40% elevation of urinary dopamine excretion. Carey et al. (1981) showed conversely that dietary sodium restriction suppressed dopamine release in man. In addition, a positive and significant correlation exists between urinary sodium and dopamine excretion under normal conditions (Cuche et al., 1972; Casson, 1984). Oates et al. (1979) further analysed the dopamine response to salt loading in man. A significant increase in dopamine excretion preceded the rise in the natriuresis

following NaCl supplementation of the diet and the authors suggested that renal dopamine synthesis may have a role in the control of salt excretion.

This effect of inorganic ions has been studied in some detail by Lee's group in the rat. Addition of excess NaCl to the diet produced a marked expected rise in urine dopamine. However, both KCl and NH_4Cl also produced significant increases, highlighting the possible importance of the chloride ion (Ball et al., 1978a). It was notable that NaHCO_3 supplementation was without effect. Dopamine is a weak base with pK 10.6 so that its excretion into urine may be pH dependent. The chloride compounds tended to acidify the urine which would limit back diffusion of dopamine from tubular fluid and increase urinary excretion. Addition of NaHCO_3 increased the pH of urine. In a further study, the effect of inorganic salts on renal tissue dopamine levels was determined (Ball et al., 1978b). Again, whereas NaCl, KCl and NH_4Cl increased renal tissue dopamine, there was no change in the group receiving NaHCO_3 , implying that the lack of urine dopamine response to the latter compound cannot be explained purely in terms of a partitioning phenomenon at the tubule. Tissue pH may therefore determine the rate of dopamine synthesis in the kidney. It is also conceivable however, that the lack of response to NaHCO_3 is spurious, as dopamine is known to be unstable in alkaline media.

Alexander et al. (1974) had also demonstrated, in women, that intravenous salt loading produced an acute rise in urine dopamine excretion. Concurrently, urine noradrenaline declined and the dopamine:noradrenaline ratio significantly increased. Thus the activity of the two catecholamines appeared to be inversely related. Such a reciprocal relationship was also noted in response to the upright posture (Cuche et al., 1972) and muscular activity (Christensen et al., 1976), reduced urine dopamine excretion occurring in the setting of increased sympathetic nerve activity and urinary noradrenaline release.

Two groups have used the dog to define further the dopamine response to intravenous volume expansion. In the first study, in spite of comparable volume expansion, isotonic saline was associated with an acute dopamine response, whereas salt-free albumin did not alter the output of dopamine (Faucheux et al., 1977). It was of note in this model, that the rise in dopamine was delayed and followed the onset of natriuresis (c.f. response to oral salt loading). In the second study, dogs were infused with hypo-, iso-, and hypertonic saline. All produced similar increases in fractional sodium excretion, but hypotonic saline did not produce a dopamine response (Cuche et al., 1983). From these studies, it is apparent that volume expansion per se is not directly related to a dopamine response in urine, but in some way, dopamine synthesis is intimately associated with the sodium and/or chloride ion. The possible flow dependent nature of renal hormone release has already been mentioned, and the question is relevant to dopamine. In man, Casson (1984) analysed 24 hour urine sample on normal diet, and whereas a significant correlation was present between sodium and dopamine, there was no such correlation between dopamine excretion and urine flow. However, in the rat, water loading was associated with an increase in urine dopamine output (Akpaffiong et al., 1980). The authors noted that urine dopamine concentration declined, and suggested that 'washout' of performed tubular luminal dopamine was responsible for the apparent rise.

Where groups have studied the effects of salt intake on dopamine release, a low salt standard diet is normally given, supplemented with NaCl as necessary, to exclude the effect of other dietary constituents. Some studies however, have specifically sought to analyse the effect of dietary intake (especially of protein) and fasting on catecholamine excretion. In the rat, dietary protein restriction produced a diminution of urine dopamine output whereas

protein supplementation of a laboratory "chow" diet stimulated release (Young et al., 1985; Kaufman et al., 1985). In a recent study in man, a protein meal stimulated urine dopamine output (Williams et al., 1986). Interestingly the natriuresis and increase in creatinine clearance in response to protein was attenuated by the presence of carbidopa leading to the suggestion that extra-neuronal decarboxylation of dopa in the kidney contributes to acute protein-induced changes in renal function. The source of the observed elevation of plasma L-dopa levels was not determined, though the L-dopa content of the meal was known to be low. Two possibilities are, an increased absorption of dietary L-tyrosine with conversion to L-dopa in the body, or endogenous release of L-dopa from an unknown site in response to protein-stimulation (discussed by Williams et al., 1986). The factors controlling plasma L-dopa concentrations may be of importance in other circumstances, but they are as yet unknown. Brown and Dollery (1981) had demonstrated a strong, positive correlation between plasma L-dopa and urine dopamine both under normal conditions and after administration of L-dopa. They therefore postulated that regulation of dopamine synthesis was secondary to influences controlling plasma L-dopa. In this regard, L-dopa administration to rats was associated with increased activity of renal dopa decarboxylase (Sato et al., 1987).

The tubular uptake of filtered L-dopa at the proximal tubule, is an active process with marked structural specificity (Chan, 1976). The transport of amino-acids at the proximal tubule has been shown to be sodium-dependent, active reabsorption declining in kidney cortex slices as the sodium concentration was lowered (Fox et al., 1964; Ullrich et al., 1974). This may represent a physiological mechanism of control which links L-dopa uptake into the proximal tubular cell, and dopamine synthesis in situ, to the filtered load of sodium. It should be noted however that L-dopa actively generated dopamine in the non-

filtering isolated perfused rat kidney, suggesting that L-dopa can also be taken up into the proximal tubular cell from the basal aspect without the necessity for glomerular filtration (Suzuki et al., 1984). Chan (1976) also demonstrated that the uptake of L-dopa was not inhibited in the presence of a dopa decarboxylase inhibitor or by large changes in pH of the perfusate.

In addition to control being exerted at the level of L-dopa uptake, it is conceivable that synthetic and degradative enzyme activity may be modulated by alterations in salt status. In rat renal tissue there was no detectable change in the activities of monoamine oxidase or dopa decarboxylase in response to salt loading (Ball and Lee, 1977b). Intravenous salt loading in women decreased plasma dopamine β hydroxylase, concurrent with the rise in urine dopamine and fall in urine noradrenaline (Alexander et al., 1974). One interpretation is that the enzyme may have a regulatory role in catecholamine synthesis, salt loading reducing the activity, thereby producing a block in the synthesis of noradrenaline in adrenergic nerves, which allows increased dopamine release. However, as already discussed, there is little evidence that free dopamine in urine derives from neuronal tissue, and it is more likely that plasma dopamine β hydroxylase levels reflect adrenergic nerve activity.

In man, the loop diuretic, frusemide, has been demonstrated to stimulate urine dopamine excretion (Kuchel et al., 1979). Similarly, in the rat, frusemide, the benzothiadiazine, hydrochlorothiazide, and the potassium-sparing agent, triamterene, all evoked significant increases in dopamine output (Akpaffiong et al., 1980). The mechanism of release is unknown. However, the study of pharmacological natriuretic agents, with actions upon different segments of the nephron, may lead to greater understanding of the factors influencing dopamine synthesis, and in particular the relationship to salt handling by the kidney.

3) DOPAMINE IN RENAL PATHOPHYSIOLOGY

Essential hypertension is a multifactorial disorder, and many hypotheses have been forwarded to account for its pathophysiological basis. Dahl's work on the rat provided evidence for a genetic component in the blood pressure response to dietary salt intake. Strains breeding true to type were produced that were either resistant to, or sensitive to, increases in salt ingestion (Dahl et al., 1962). Cross-transplantation experiments indicated that the basis for differential responses lay in the kidney (Tobian et al., 1966).

In man, essential hypertension is believed to have a genetic component, with a polygenic mode of transmission, in which environmental influences act upon an inherited background susceptibility.

Hall (1987) has drawn attention to the importance of renal excretory capacity in the long-term regulation of arterial pressure: any impairment will result in an increase in blood pressure to maintain sodium and water balance. De Wardener and MacGregor (1982) have proposed that a primary defect in renal salt excretion exists in essential hypertension, sodium retention leading to the compensatory release of a natriuretic factor, the Na^+/K^+ -ATPase inhibitor from the hypothalamus. The nature of the renal defect remains unknown.

The relationship between dietary salt intake and the prevalence of essential hypertension in man is a subject of great controversy (reviewed by Houston, 1986). Part of the explanation for conflicting results, derived from cross-cultural comparisons on the one hand, and controlled intervention studies on the other, may lie in the heterogenous nature of the disease. By means of either chronic elevation of dietary salt intake, or acute intravenous salt loading, subgroups of individuals have been identified who either show a pressor

response to salt, 'salt-sensitive' subjects, or who are able to tolerate large increases in salt intake with apparent impunity, salt-resistant subjects (Fujita et al., 1980; Weinberger et al., 1986).

Dopamine is both natriuretic and renal vasodilator on administration, and may have a physiological role in renal salt handling. There has therefore, been interest in assessing endogenous dopamine in essential hypertension. Dopamine excretion into urine was shown to be reduced in a group of essential hypertensives, and the acute dopamine response to frusemide was impaired (Kuchel et al., 1979). Harvey et al. (1984) studied a group of essential hypertensives with no evidence of renal damage. Contrary to the expected result, they displayed a paradoxical and inappropriate decline in dopamine excretion in the face of a salt load. This work has been followed up by Shikuma et al. (1986). A group of patients with essential hypertension were defined as salt-sensitive or salt-resistant in terms of a pressor response to salt loading. They demonstrated that the salt sensitive group did not mobilise dopamine whereas the salt-resistant group produced the expected response. This defect appears to have a genetic basis and antedates the development of hypertension: the normal correlation between dopamine and sodium excretion was lost in a Japanese population of normotensive first-degree relatives of patients with essential hypertension (Saito et al., 1986). Patients with essential hypertension, in addition, have increased renal responsiveness to low dose dopamine infusion, which is manifest in an exaggerated rise in RBF and natriuresis and an enhanced hypotensive effect in comparison to normal controls (Andrejak and Hary, 1986). One interpretation of such results is that a relative deficiency of dopamine leads to upregulation of dopamine receptors in the kidney, and a greater response to exogenous dopamine. Evidence to date therefore suggests that some individuals are prone to the hypertensive effect of excess dietary



salt intake, and an inherited inability to mobilise dopamine in the kidney may, at least partially, account for the underlying renal defect. It should be appreciated that other factors have been advocated, including kallikrein and the renal prostaglandins (Weber et al., 1979; Warren and O'Connor, 1980).

The potential evolutionary advantage for effective sodium retention mechanisms has been alluded to. Black populations in urban societies have a greater prevalence of essential hypertension. In addition, essential hypertension with renin suppression is more common, suggesting primary volume overload (Warren and O'Connor, 1980). Negroes exhibit a greater pressor response to increases in dietary salt and excrete sodium less effectively after intravenous loading (Luft et al., 1977; 1979). Critchley et al. (1987) has studied a group of normotensive Ghanaians living in Edinburgh. As opposed to a Caucasian control group, they did not display the normal correlation between urinary sodium and dopamine, and did not produce a dopamine response to salt loading. It would appear that blacks are less able to deal with the large salt intakes of westernised society. It may be that renal mobilisation of dopamine is poorly developed as a natriuretic mechanism in blacks, and that the balance of forces is weighted towards salt retention commensurate with their original hot and humid environments.

As chronic renal failure advances with progressive nephron loss, the absolute ability of the kidney to excrete sodium declines. Hypertension is an inevitable consequence of sodium retention and increase in total body sodium and water content (Brennan et al., 1980; Koomans et al., 1981). Itskovitz and Gilberg (1981) showed that with increasing renal failure, urinary dopamine diminished and almost disappeared when plasma creatinine concentration was greater than 530 $\mu\text{mol/l}$. In a group of patients with chronic glomerulonephritis and mean plasma creatinine of 240 $\mu\text{mol/l}$, urine dopamine excretion was

reduced by more than 50%, and there was no dopamine response to elevation of dietary salt intake (Casson et al., 1983). The authors speculated that the loss of normal control of dopamine synthesis may be a factor in the abnormal retention of salt and water.

There is no information as to whether impairment of endogenous dopamine release has a pathophysiological role in the development or maintenance of acute renal failure. Although it is a heterogeneous condition, which can be initiated by diverse insults, studies in animals have pointed to the early importance of vasoconstriction in the kidney (reviewed by Paller and Anderson, 1983). Low dose dopamine infusion is widely used in the setting of incipient renal failure where promotion of renal vasodilatation and natriuresis may prevent the onset of acute tubular necrosis. In addition, one or two studies have suggested that the combination of dopamine and frusemide may be of benefit in established acute renal failure. Conversion to non-oliguric failure was demonstrated in a proportion of patients and in some this was associated with functional recovery (Henderson et al., 1980; Lindner, 1983).

4) γ -L-GLUTAMYL-L-DOPA AS A RENAL PRODRUG

The potential of dopamine as a therapeutic agent was first suggested by the study of McDonald and colleagues in 1964. In a group of patients with congestive cardiac failure, infusion at low dose resulted in renal vasodilation and natriuresis, and an increase in the cardiac index. Since that time, it has become widely used in the intensive care setting, particularly in cardiogenic shock and incipient acute renal failure. Dopamine is not specific for dopaminergic receptors, but activates both α and β adrenergic receptors depending on the dose infused. Between 1 and 4 $\mu\text{g/kg/min}$ its action is primarily on dopaminergic receptors, producing systemic and renal vasodilation and a small, variable reduction in blood pressure. β receptors are stimulated maximally between 5 and 10 $\mu\text{g/kg/min}$, accounting for the drug's inotropic and chronotropic effects. Above this dose, α effects predominate with systemic vasoconstriction (Goldberg, 1972). Thus the dose range for dopaminergic actions is narrow, and pedal gangrene resulting from intense vasoconstriction has been reported with its use (Alexander et al., 1975). In addition, individuals may show abnormal sensitivity to the drug: a patient developed gangrene during the prolonged infusion of only 1.5 $\mu\text{g/kg/min}$ (Greene and Smith, 1976).

There has therefore, been interest in the evaluation of agents producing the beneficial effects of dopamine receptor stimulation, whilst avoiding the potentially damaging consequences of activation of α and β receptors. One approach has been the development of agonist drugs with relative specificity for dopamine receptors.

DA_1 receptors are widely distributed in the vasculature, activation resulting in systemic and renal vasodilatation, and reduction in blood pressure. DA_2 receptor agonists have a similar action, inhibition of noradrenaline release

allowing passive vascular relaxation. Both DA₁ and DA₂ agonists are currently being assessed for efficacy in essential hypertension and congestive cardiac failure (Goldberg, 1984; Harvey et al., 1986; Clark, 1987). There are, however, disadvantages with both groups. The action of DA₁ agonists, like other vasodilators, leads to secondary activation of the sympathetic nervous system with tachycardia and offsetting of drug action. DA₂ agonists do not increase the pulse rate, but their use is associated with nausea due to stimulation of the chemoreceptor trigger zone at the area postrema. In addition, those passing the blood-brain barrier such as lisuride, may produce a dose-dependant psychosis (Critchley et al., 1986).

Another approach has been to focus on the renal actions of dopamine, namely vasodilatation and natriuresis, and to develop drugs with effects targeted at, and limited to, the kidney. As discussed, the basic defect in at least a proportion of those with essential hypertension, may reside at the kidney, with a relative inability to excrete salt. Targeting dopaminergic drug action at the kidney may allow a smooth and sustained reduction in blood pressure concurrent with the natriuresis. Similarly, in congestive heart failure and incipient renal failure, promotion of natriuresis is desirable without acute and marked fluctuations in systemic blood pressure.

The physiological precursor of dopamine is L-dopa, and its administration in man results in an increase in renal blood flow and sodium excretion. However, it also produces changes in systemic haemodynamics, and its use is associated with undesirable side-effects, partly resulting from its propensity to cross the blood-brain barrier (Finlay et al., 1971; Rajfer et al., 1984).

Goldberg's group first developed the concept of the renal prodrug (Biel et al., 1973). They demonstrated that the aminoacyl derivative of dopamine (N-L-isoleucyl-dopamine) produced a selective increase in renal blood flow with

only minimal systemic haemodynamic effects in the anaesthetised monkey. The basis of the relative specificity was due to conversion to dopamine by the enzyme aminoacylarylamidase which is abundant in the kidney.

The work of Orlowski and Wilk (1976, 1978) indicated that the kidney is active in the uptake and metabolism of γ -glutamyl derivatives of amino-acids and peptides. The activity is related to the presence in the kidney of the enzyme γ -glutamyl transferase, shown by histochemical techniques to be highly concentrated in the brush border of proximal tubular cells in rats (Albert et al., 1961; Glenner et al., 1962) and in man (Abbs and Kenny, 1983). The enzyme has also been demonstrated at the antiluminal surface in association with the microvasculature, and the high concentration of enzyme at the proximal tubule suggests an important physiological role in amino-acid handling, glutathione transport and metabolism, and renal ammoniagenesis (Welbourne and Dass, 1982). In the human, if activity in the kidney is taken as 100, relative activities in other tissues are; pancreas - 8.3, liver - 3.9, spleen - 1.5, intestine - 0.95, brain - 0.5, lung - 0.31, skeletal muscle - 0.067, and heart - 0.045 (Orlowski and Szewczuk, 1961).

Wilk and colleagues (1978) administered γ -glutamyl-L-dopa to rats and mice by intraperitoneal injection and noted marked concentration of dopamine in renal tissue, considerably higher than in other tissues. For example, the ratio in kidney to that in heart was 25.8. After administration of L-dopa at an equimolar dose, a much more uniform distribution pertained, and the ratio was 4.3. It had previously been reported in guinea pigs that the kidney/heart ratio after intravenous dopamine was just 2.5 (Halushka and Hoffman, 1968).

The differential effect of dopamine accumulation was also reflected in renal blood flow. Gludopa produced renal vasodilation whereas an equimolar dose of L-dopa was without effect. Another index of specificity was the relat-

ive increase in dosage required to elevate systemic blood pressure above that for renal vasodilation. A ten-fold increase in dopamine elevated both systolic and diastolic pressure, whereas a 20-fold increase was required for gludopa, the rise being principally in the systolic component. The same group have also evaluated another derivative, γ -glutamyl dopamine. It proved more renally selective than dopamine, but less potent than gludopa.

The presence of γ GT on both luminal and antiluminal membranes suggests that the uptake of gludopa will be very efficient and occur both after filtration, from tubular lumen, and from the renal circulation. It is likely that the enzyme functions both as carrier and hydrolytic unit so that free L-dopa is liberated into the proximal tubular cells. L-dopa will then be available for conversion to dopamine under the action of dopa decarboxylase, which as discussed, is present in high concentration in proximal tubular cells (Figure 2). The renal specificity of gludopa thus depends on the consecutive action of two enzymes, both concentrated in the kidney. Since the generation of dopamine from γ -glutamyl dopamine requires the action of only one enzyme, it would be expected to be less renally specific than gludopa.

Worth and colleagues (1985a, 1986) first infused gludopa into man, and established its natriuretic potency at 12.5 and 100 $\mu\text{g/kg/min}$. In normal volunteers, it produced renal vasodilation with a small increase in glomerular filtration rate and reduction in filtration fraction. At the lowest dose it was devoid of effects on systemic haemodynamics, although at 100 $\mu\text{g/kg/min}$ there was a small reduction in diastolic blood pressure and a small increase in pulse rate. Its effectiveness as a renal prodrug with relative renal specificity was demonstrated by the 280-fold and 2500-fold increases in urine dopamine excretion at low and high doses, whereas plasma dopamine levels rose to only 4 and 25 times the baseline levels, respectively.

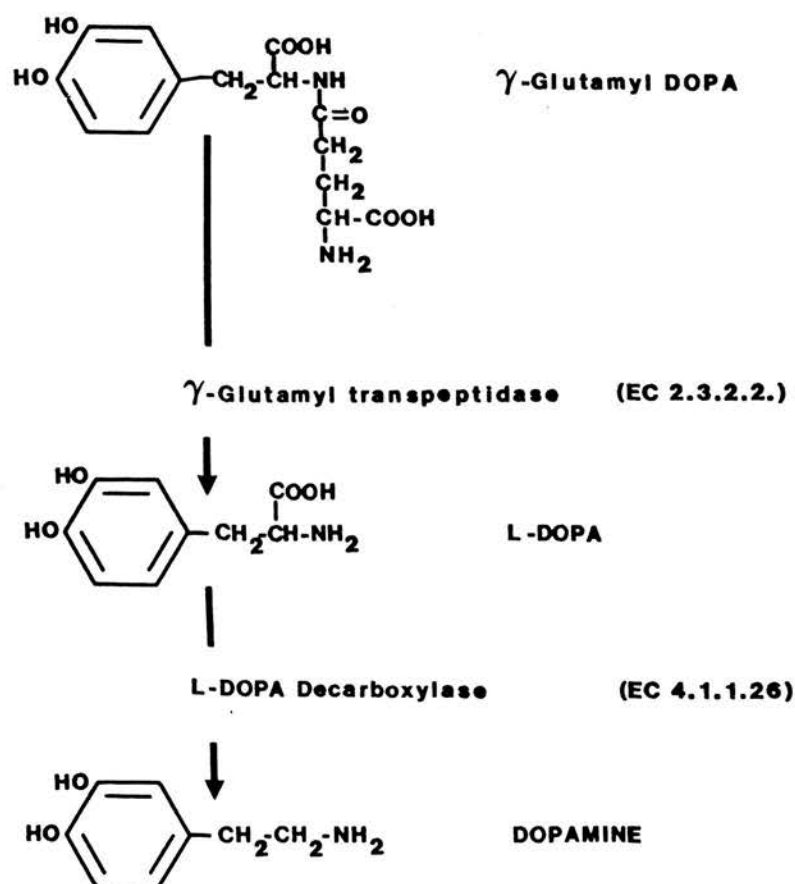


FIG. 2. The conversion of gludopa to dopamine by sequential removal of the glutamyl and carboxyl radicals.

The agent has at present been used in two pathological states. Casson (1984) administered glycerol which induces an ischaemic model of acute renal failure in the rat. When gludopa was administered concurrently by subcutaneous route, the pathological and clinical sequelae were significantly alleviated. Gludopa has also been infused into human patients with essential hypertension (Worth et al., 1985b). In comparison with controls, there was an exaggerated natriuretic response to gludopa, similar to the findings with infused dopamine.

CHAPTER TWO

METHODS

1) VOLUNTEER SELECTION AND GENERAL PROTOCOL OF THE STUDIES

Volunteers were healthy normotensive males aged 19 - 40 years. A health screen was performed including general examination, supine blood pressure, routine biochemistry and full blood count, urinalysis and electrocardiogram. Subjects were excluded on the basis of previous drug reaction, allergy, or requirement for drug therapy. Women were not included in view of the possibility of first trimester pregnancy, and the known effects of the oral contraceptive pill on salt and water balance. All volunteers gave informed consent and the studies were subject to approval by the Medicine and Clinical Oncology, Ethics of Medical Research Subcommittee, Lothian Health Board.

To reduce the variability of responses, subjects were given advice to avoid excessive dietary salt, by avoiding salty and highly processed foods and not adding salt to food over the 36 hours prior to a study. Alcohol was forbidden for 36 hours prior to the study and caffeine containing drinks (tea, coffee, coke, chocolate) and smoking were not allowed from 2200 hours on the evening before. Subjects avoided all proprietary medicines including aspirin for at least one week before investigation. On the morning of study, 500 mls of tap water and a light breakfast were taken at 0800 hours before arrival at the investigation suite by 0900 hours. Subjects remained supine throughout, except for bladder emptying. Intravenous Venflon cannulae were placed in either or both antecubital fossae for blood sampling and drug administration. Water (200 ml/hr) was given frequently by mouth to ensure adequate urine flow rates and to facilitate complete bladder emptying. In all studies, a run-in or baseline period was followed by drug administration, either orally, or by intravenous bolus or infusion, and the study then continued for a variable period with a

recovery phase as indicated. All drug infusions were given with an Imed 960 Volumetric pump. Sampling was performed at timed intervals such that, at the end of a collection period, supine pulse and blood pressure measurement, and blood sampling, were followed by standing and voiding. Blood pressure and pulse were measured in duplicate by automatic inflating cuff (Critikon Dinamap).

Studies were separated by at least 7 days to allow washout of drugs and re-equilibration of salt balance and renal responses. Unless otherwise specified, the studies were carried out in a random-ordered, single-blind fashion. Specific details for each particular study are discussed in the relevant chapters.

2) DRUGS

γ -L-glutamyl-L-dopa (gludopa) was obtained from UCB Bioproducts (Brussels). The preparation used in these studies contained 92% dipeptide, the balance being acetic acid carried over from freeze-drying. It was prepared for use in sterile ampoules containing 100 mg of gludopa in 5 ml of 0.9% sodium chloride solution with 0.5% human albumin. Before use, the required amount was added to 50 mls of 0.9% sodium chloride to give 25 μ g/kg/min on intravenous infusion over the specified period.

γ -L-glutamyl-L-tyrosine (glutytrosine) was obtained from Sigma. It was prepared in the same manner as for gludopa.

Carbidopa was kindly donated by Merck Sharp and Dohme Ltd.

3) ASSAYS

URINE FREE DOPAMINE

Introduction

Up to 80% of urine dopamine may be in conjugated form, principally the sulphoconjugate (Kuchel et al., 1979). As discussed in the introduction, the physiological moiety is likely to be free dopamine. In the present assay, urine samples were initially extracted with alumina, the rationale being that only free dopamine binds to alumina, which can then be eluted. The extraction phase is based on the method of Anton and Sayre (1964). Thereafter, free dopamine was measured by high performance liquid chromatography (HPLC) and electrochemical detection, using epinine (N-methyl dopamine) as an internal standard.

Collection of urine samples

Timed urine samples were collected into 25 ml Universal Containers with 0.4 ml of 5M HCl. This was sufficient to lower the pH of the samples to below 3, which prevents oxidation of free dopamine. Aliquots were stored at -40°C before assay.

Reagents

Dopamine HCl was obtained from Sigma. A stock solution was prepared by adding 50 mg to 50 ml M HCl (1 mg/ml). The working standard was prepared by diluting the standard 1:100 with distilled water and further diluting 1:50

with 0.01 M HCl to produce a concentration of 200 ng/ml.

Epinine was obtained from Sigma. The stock solution was prepared by adding 50 mg to 50 ml of M HCl. It was stored in aliquots at -40°C . The working internal standard was prepared by diluting the stock 1:10 with distilled water and further diluting 1:50 with 0.01 of M HCl to produce a concentration of 2 $\mu\text{g}/\text{ml}$.

The solvent used for HPLC contained citric acid monohydrate (5.75g), sodium acetate trihydrate (6.80g), sodium hydroxide (2.40g), 1-octane sulphonic acid, sodium salt (0.10g), acetic acid (1.05ml), di-sodium EDTA (0.10g) made up to one litre of deionised water. The pH was 5.2. Solvent was filtered through a 0.22 μm membrane (Millipore), and methanol 10% (v/v) added prior to use. Dissolved oxygen was removed by bubbling helium through the solvent.

Aluminium oxide (Brockman grade 1, B.D.H.) was pre-activated by heating at 200°C for 2 hours and stored at 100°C . Activated alumina was washed with 0.5M EDTA before use.

Chemical reagents used were obtained from standard suppliers.

Extraction of dopamine from urine samples and standards

The volume of urine used depended upon the likely concentration expected. For example, in studies involving the administration of gludopa, considerable dilution such as 1:200 with distilled water was necessary.

To 0.5 g of washed activated alumina, were added 5 ml of the test urine sample and 0.5 ml of the working internal standard. The pH was adjusted to 8.3 with 0.5 M Tris. The mixture was rollmixed for 40 mins at 37°C and centrifuged at 1000 rpm for 2 mins at 4°C , before syphoning off the supernatant. The deposit was washed three times with distilled water, each time syphoning off the supernatant after centrifugation. Dopamine was eluted by addition of 2

ml, 0.2 M acetic acid. Tubes were shaken for 10 minutes at 37°C and centrifuged at 4°C. The supernatant was removed by means of a Porex filter, and stored at -40°C. All samples were processed and analysed in duplicate.

Dopamine standards were prepared in two ranges, 20 - 200 ng/ml, or 5 to 50 ng/ml depending upon expected sample levels. 0.5 ml of internal standard, 2 µg/ml or 0.5 µg/ml, was added to 5 ml of each standard concentration. All standards were extracted following the same procedure as that for test samples, and frozen at -40°C. Sufficient standards were prepared for one months use.

The recovery of dopamine from the extraction process was $69.7 \pm 0.5\%$ S.D. as compared against unextracted standards.

Analytical System

Each assay consisted of test samples in duplicate standards, run at the beginning and end of each batch, and two quality control samples. 5 µl were injected automatically by a Waters Intelligent Sample Processor, into a solvent stream delivered at 1 ml/min by a Beckman/Alter M410 solvent metering pump. Solvent thereafter passed to a Waters 4 µm, Novapak C18 HPLC column under radial compression and the column effluent stream to a Waters 460 electrochemical detector, set at a potential of 0.4 volts, in oxidative mode, with background current approximately 0.5 mA. Signals were analysed by a Shimadzu CR3A integrator which measured the area under the curves of peaks of interest.

Expression of results

The integrated area of all standard peaks was divided by the area of its own internal standard. The duplicate standard ratios were measured and a

standard curve plotted against known concentrations, the plot providing a straight line relationship. Test sample ratios were read from the curve and urine dopamine expressed in nmol/l.

Intra- and inter-assay co-efficients of variation were 4% and 6% respectively. The sensitivity of the assay was 5 ng/ml.

PLASMA RENIN ACTIVITY

Introduction

Renin, a proteolytic enzyme of the aspartyl protease class, has as its natural substrate angiotensinogen - an α_2 macroglobulin, synthesised in the liver and released into the circulation. It cleaves off a decapeptide, angiotensin I which is rapidly converted in vivo into angiotensin II, under the action of a dipeptidylcarboxypeptidase, angiotensin converting enzyme. Angiotensin II is biologically active with a half-life of 2-3 minutes in vivo, and is reduced to small peptides by angiotensinases. Incubation of plasma in the presence of powerful inhibitors of angiotensinases and converting enzyme activity and assay of generated angiotensin I by radio-immunoassay allows measurement of plasma renin activity. The method used is based on that developed by Haber et al. (1969).

Plasma renin activity is influenced by posture and stress; therefore, sampling was performed after subjects had been quietly supine for at least 20 minutes. In addition, inactive renin, currently termed pro-renin, can be activated by exposure to cold (-5°C to $+4^{\circ}\text{C}$); therefore samples were stored at -40°C and assayed on ice (4°C).

Reagents

Antibody to angiotensin I was raised in the rabbit and kindly provided by Dr. Frank Goodwin (London Hospital). It was prepared for use as a 1:20 000 dilution in 0.1 M Tris-acetate buffer (pH 7.4) containing 0.25% bovine serum albumin (BSA) to give 50% binding to labelled angiotensin I.

Iodine¹²⁵ - labelled angiotensin I was obtained from CIS(UK). Sufficient crystals were dissolved in Tris-acetate buffer with 0.25% BSA (pH 7.4) to give between 1500 and 2000 cpm/200 μ l.

Standard angiotensin I was obtained from the National Institute of Biological Standards and Control. The working standard was 180 pg/100 μ l, double diluted using 500 μ l of Tris-acetate buffer with 0.25% BSA (pH 7.4) to give a range of 180, 90, 45, 22, 11, 5 pg/100 μ l for construction of standard curves.

The inhibitor mix consisted of phenyl-methyl-sulphonyl fluoride 5% in ethanol (2 parts), dimercaprol, 1.06 M solution in ethanol (one part) and 8-hydroxyquinoline, saturated solution in ethanol (one part).

The chemical reagents used were obtained from standard suppliers.

Method

Venous blood (5 ml) was collected into pre-cooled tubes containing 100 μ l of a 5% sodium EDTA solution and kept on ice until centrifugation at 4°C, separation and storage of plasma at -40°C.

40 μ l of phosphate buffer (pH 5.3) and 10 μ l of inhibitor mix were added to duplicate 200 μ l aliquots of plasma samples. One set was incubated for one hour at 37°C in a water bath during the generation phase, the other set remained at 4°C to allow measurement of any angiotensin I generated during collection, together with any substances cross-reacting with the antibody. The

reaction was terminated by the addition of one ml of cold water.

To 100 μ l aliquots of the duplicate samples, were added 400 μ l Tris-acetate buffer containing 0.5% BSA and 0.1% lysozyme chloride, 200 μ l of antibody and 200 μ l of angiotensin I label. Standards were prepared in duplicate in the same manner. In addition, tubes were prepared containing antibody and label only to check binding and provide the zero point on the standard curve, and two extra tubes contained only buffer and label as a background check for how much non-angiotensin antibody was present. All samples were left for 20-24 hours at 4°C.

The principle of charcoal separation is that free angiotensin I binds to dextran-coated charcoal whereas the angiotensin-antibody complex does not. The efficiency of separation is highly time and temperature dependant and requires precise control. 0.9 g charcoal and 0.1 g dextran (mol.wt. 81000) were dissolved in 50 mls Tris-acetate buffer with 0.25% BSA and mixed on ice for 45 minutes. Over a timed period of 9 minutes, 500 μ l was added to each tube, except total count tubes, mixed and left on ice. Batches were then spun at 3000 rpm for 10 minutes at 4°C. The decanted supernatant was counted for 10 minutes using a LKB-Wallace 1275 Mini Gamma gammacounter. The "bound counts" were expressed as a percentage of total counts, and by plotting a standard curve for the known angiotensin I concentrations, the sample values could be read directly from the curve. The 4°C incubation value was subtracted from each sample, the result multiplied by a dilution factor of 0.0625 and plasma renin activity expressed as ng angiotensin I/ml/hr. Intra-assay and inter-assay co-efficients of variation were 4% and 6% respectively.

ATRIAL NATRIURETIC PEPTIDE

Introduction

Many peptide fragments with natriuretic activity have been identified from atrial extracts in animals and humans. It would appear that a 126-amino-acid prohormone, stored in granules in perinuclear atrial cardiocytes undergoes selective enzymotic cleavage on secretion, releasing the 28-amino-acid carboxy-terminal fragment now called α -hANP. This peptide is believed to be the normal circulating form of the hormone in man.

ANP was measured in plasma samples in the present studies by a radio-immunoassay, after an extraction phase, using rabbit antiserum raised to synthetic α -hANP.

Posture is known to affect plasma ANP levels: therefore subjects remained supine for at least 20 minutes before sampling. Lipid-laden plasma samples interfere with the extraction procedure and subjects either fasted before sampling or took only a light breakfast, depending on the study in question.

Materials

α -hANP antibody was raised in this department. 1-28 α -hANP (Peninsula 0.5 mg in 1 ml sterile 0.15 M NaCl) was added to a sterile mixture of bovine thyroglobulin (5 mg) and carbodiimide (3 mg in 3 ml sterile 0.15 M NaCl). The solution was left overnight at 4°C before a further 3 mg of carbodiimide was added. It was stirred at room temperature for 3 hours and dialysed in $\frac{1}{4}$ inch Visking tubing against 5 l of 0.15 M NaCl at 4°C overnight. Thereafter, the solution was made up to 5 ml with sterile 0.15 M NaCl. One ml of the conjugate was mixed with 1 ml complete Freund's adjuvant, and injected intra-

muscularly into a New Zealand white rabbit, 0.5 ml at each of four different sites. At four 3 weekly intervals, the remaining conjugate was administered subcutaneously, 0.5 ml at two sites, on each occasion. The rabbit was bled from the ear artery from the 6th week onwards, at weekly intervals, and bled out when the antibody titre showed 50% binding at a 1:11000 dilution. The serum was stored at 1:500 at -40°C and diluted to give a working concentration of 1:11000.

Iodine¹²⁵ - α -hANP label was obtained from Amersham International. It was stored at -40°C as aliquots containing 500 pg in 500 μl and diluted for use to 4 pg/100 μl .

Standard α -hANP was obtained from Bachem. It was stored at -40°C as aliquots containing 200 pg/100 μl . A range of 5 - 200 pg/100 μl was produced by double dilution.

Filtered phosphate buffer 0.1 M, pH 7.4 containing 0.25% egg albumin, was used for all dilutions.

The chemical reagents used were obtained from standard suppliers.

Method

Venous blood (10 mls) was collected into pre-cooled plastic tubes containing 5% disodium EDTA and Trasylol (20 KIU/ml) to prevent destruction of ANP by proteases, and kept on ice until centrifugation at 4°C , separation and storage of plasma at -40°C .

Extraction of samples was performed using a SEP-PAK C₁₈ cartridge pack (Waters Associates) and vacuum source to pull solvents through. Each column was wetted with 4 ml methanol and washed twice with 4 ml of 4% acetic acid. Four ml of plasma was applied to the column and washed with 4 ml of 4% acetic acid. The ANP was eluted from the column using 2.5 ml of

ethanol solvent (ethanol 80%, acetic acid 4%). The eluate was dried under a stream of N_2 at $37^\circ C$ and the residue reconstituted in 400 μl of buffer and stored at $-40^\circ C$.

During the assay procedure, 100 μl of sample was added to 600 μl of buffer and 100 μl of antibody. The mixture was incubated at $4^\circ C$ for 24 hours. Thereafter, 100 μl of ANP label was added and the mixture incubated for a further 20 hours at $4^\circ C$. Standards were prepared in a similar manner, and extra tubes set up for non-specific binding. Dextran coated charcoal was prepared by adding 0.08 g dextran (mol at 81000) and 0.8 g charcoal to 50 ml buffer and mixing on ice for 45 minutes before use. 500 μl was added to all samples, standards and controls (except total count tubes), the procedure being performed in batches of 40 tubes over 10 minutes. Tubes were then spun for 15 minutes at 2500 rpm, decanted and the supernatant counted over 10 minutes using a LKB-Wallace 1275 MiniGamma gammacounter. In the same way as for the PRA assay, "bound counts" were expressed as a fraction of total counts, and values read off a standard curve. Values are expressed as pg/ml after correction for extraction and dilution during the assay. Recovery from the SEP-PAK cartridge of I^{125} - α -hANP was $89.5\% \pm 0.58\%$ S.D. Intra-assay and inter-assay coefficients of variation were 4.0% and 8.4% respectively.

URINE KALLIKREIN

Introduction

Glandular kallikrein, produced by the kidney and excreted in urine, is a serine protease, identical to that found in salivary and sweat glands, pancreas and gastro-intestinal tract of the same species, but distinct from plasma kalli-

krein. It acts on acidic glycoproteins, kininogens, to release lysylbradykinin which has potent actions in the kidney.

Urine kallikrein was measured in the present studies by the method of Amundsen et al (1979). Kallikrein in urine hydrolyses the chromogenic substrate H-D-valine-leucine-arginine-p-nitroaniline: the rate of p-nitroaniline released increases linearly with increasing concentration of kallikrein and can be measured spectrophotometrically. In view of the highly diluted urine produced by water loading and diuretic administration, urine samples were initially concentrated by ultrafiltration.

Reagents

Substrate S-2266 (H-D-Val-Leu-Arg-pNA 2HCl) was obtained from Kabi Diagnostica, Stockholm. A 1.5 mmol/l solution in distilled water was stored at 4°C.

Tris buffer, 0.2 mol/l, pH 8.2, was used for all dilutions.

Standard porcine kallikrein (Sigma) was stored as 1 Eu/100 µl aliquots in 0.9% NaCl. A working range of 0.01 to 0.04 Eu/ml was prepared with distilled water.

The chemical reagents used were obtained from standard suppliers.

Method

Urine samples were stored at -20°C. Samples were concentrated before assay by a factor of 5-20, by means of vacuum-operated Immersible-CK Ultra-filters (Millipore) containing 10,000 molecular weight cut-off membranes. The concentrated urine was then brought back to 5 ml with distilled water used to rinse the membrane.

For the assay, 400 µl of concentrated urine was added to 500 µl of

buffer and kept for 5 minutes at 37°C, before addition of 100 µl of S-2266 solution. The mixture was incubated for 30 minutes at 37°C. Thereafter tubes were transferred to ice and 100 µl of 50% acetic acid added. Standard samples were treated in the same manner. In addition, a parallel line of urine samples were analysed using buffer containing Trasylol (lyophilised aprotinin 20 KIU/ml), a potent inhibitor of glandular kallikrein. In such sample blank tubes, protease activities not inhibited by aprotinin as well as the colour of the urine itself could be estimated. The absorbance of standards, samples and blanks were read at 405 nm by spectrophotometer (Pye/Unicam SP 1750). The sample blank values were subtracted from those of the sample tests and the concentration of kallikrein in samples read from the constructed standard curve. The original urine kallikrein concentrations in EU/ml were calculated after correction for a variable concentration factor. Recovery during the concentration procedure was $95.4 \pm 8.5\%$ S.D. The intra- and inter-assay co-efficients of variation were 3.7% and 5.1% respectively.

Urine cyclic AMP

Urinary cAMP was kindly measured by Dr. P.W.H. Rae, Department of Clinical Chemistry, The Royal Infirmary, Edinburgh. The method was a radio-immunoassay with a rabbit anti-BSA-linked cyclic AMP antibody. Separation was achieved using a donkey anti-rabbit serum attached to Sepharose. The sensitivity of the assay was 2.6 nmol/l and the intra- and inter-assay co-efficient of variation were 6% and 10% respectively (O'Reilly et al., 1986).

Plasma and urine sodium and potassium were measured by a Corning 435 flame photometer with Corning 800 diluter.

Plasma and urine creatinine were measured by multichannel analyser (Gemstar,

Electro-nucleonics Inc.) utilising the Jaffé reaction. Briefly, when a sample is added to an alkalinised picric acid solution, the colour change read spectrophotometrically at 500 nm, against a reagent blank, is linearly related to creatinine concentration.

Urine phosphate and urate were measured by continuous flow multichannel analyser (Technicon SMA-2).

Serum and urine lithium were measured by Jenway PFP7 digital flame photometer with lithium specific filter.

4) CALCULATIONS AND STATISTICS

Creatinine clearance (C_{Cr}) as an approximate measure of glomerular filtration rate, is defined as:

$$C_{Cr}(\text{mls/min}) = \frac{U_{Cr} \times V}{P_{Cr} \times t}$$

where U_{Cr} is urine creatinine concentration ($\mu\text{mol/l}$), V is urine volume over a timed collection period (t) in minutes, and P_{Cr} is plasma creatinine concentration ($\mu\text{mol/l}$).

Lithium and sodium clearances can be similarly calculated. Clearance periods were between 45 and 60 minutes. Plasma creatinine, sodium and lithium were measured at the start and end of the period and the mean value taken for estimations.

Fractional lithium clearance is the fraction of the filtered lithium which is excreted in the urine and can be calculated as:

$$\text{Fractional } C_{Li} = \frac{C_{Li}}{C_{Cr}} \times 100\%$$

Of the sodium leaving the proximal tubule, that proportion which escapes reabsorption at the "distal tubule" (loop of Henlé, distal convoluted tubule and collecting ducts) can be represented by the following formula:

$$\text{Fractional "distal tubular" clearance} = \frac{C_{Na}}{C_{Li}} \times 100\%$$

Absolute reabsorption of sodium by the "distal tubule" can be calculated as:

$$\text{Absolute distal reabsorption (mmol/min)} = (C_{\text{Li}} - C_{\text{Na}})P_{\text{Na}}$$

Results are expressed as means \pm SEM. Data was analysed by repeated measures analysis of variance (Genstat, Rothampsted Experimental Station). Most analyses compared responses at time points after some intervention, with results for a control period, and Dunnett's correction for multiple comparisons was used (1955). Where single comparisons were made, student's t-test for paired samples was employed. The values for PRA were log-transformed before analysis in order to normalise the data. All tests were two-tailed and results of $p < 0.05$ were taken as statistically significant.

CHAPTER THREE

STUDIES WITH γ -L-GLUTAMYL-L-DOPA (GLUDOPA) IN NORMAL MAN

INTRODUCTION

The work from Wilk's group (1978) in the rat had suggested that gludopa was a promising dopamine prodrug with relative renal specificity. Worth (1986) followed up these findings by infusing the dipeptide in man, and showed it to be active in terms of natriuresis and renin suppression when infused at 25 and 100 $\mu\text{g}/\text{kg}/\text{min}$. At the higher dose however, there were systemic effects with slight reduction in blood pressure, elevation of the pulse rate, and nausea in some subjects.

The three studies in this chapter were devised to extend this work and further characterise the action of gludopa in the kidney. In addition to a potential role as a therapeutic agent, a renally specific drug such as gludopa provides a useful tool to investigate natriuretic mechanisms without the confounding effects of changes in the cardiovascular system, and may allow actions and interactions of dopamine in the kidney, under physiological conditions, to be inferred. An infusion rate of 25 $\mu\text{g}/\text{kg}/\text{min}$ was chosen with the aim of optimising renal effects of gludopa without producing appreciable changes in the systemic parameters, blood pressure and pulse.

The uptake of L-tyrosine is an active process, and the formation of L-dopa, catalysed by the enzyme tyrosine hydroxylase, is the rate limiting step in catecholamine synthesis in peripheral adrenergic nerves (Nagatsu, 1973). Dopamine synthesis has been demonstrated extraneuronally in the proximal tubule in the kidney (Hagege and Richet, 1985) and evidence reviewed suggests that, under normal conditions, renal dopamine is formed from the uptake and decarboxylation of circulating L-dopa (Lee, 1986). The first study compared the urine dopamine synthesis and renal actions following infusion of the dipeptides γ -L-glutamyl-L-dopa and γ -L-glutamyl-L-tyrosine. With the known abund-

ance of γ -glutamyl-transferase in the kidney (Albert et al., 1961), the release of large amounts of free L-dopa and L-tyrosine would be expected. The aim of the study was therefore to compare the relative effectiveness of L-tyrosine and L-dopa as substrates for dopamine synthesis in the kidney.

The second study was composed of two parts. The effect of the peripheral dopa decarboxylase inhibitor, carbidopa, on the renal response to gludopa, was assessed to characterise the enzyme requirement for its action. Indomethacin, a cyclooxygenase inhibitor was administered to investigate interactions of dopamine, formed after infusion of gludopa, with the prostaglandin and kallikrein-kinin hormonal systems in the kidney.

Lithium is reabsorbed with sodium at the proximal tubule and its renal clearance has been proposed as a quantitative marker for sodium reabsorption at this segment of the nephron (Thomsen, 1984). The lithium clearance method provides an attractive in vivo technique for the assessment of a direct proximal tubular effect of dopamine, after the infusion of gludopa. However, lithium has itself been demonstrated to alter renal electrolyte handling (Singer, 1981) and during preliminary studies it was suspected that lithium was interfering with the natriuretic capacity of gludopa. The third study was undertaken to further characterise any interaction between lithium and dopamine in the kidney.

1(a) A COMPARISON OF THE RENAL ACTIONS OF γ -L-GLUTAMYL-L-DOPA
AND γ -L-GLUTAMYL-L-TYROSINE

Protocol

Six volunteers were each studied on two occasions. Each day consisted of three 2 hour periods (-2-0, 0-2, 2-4). During the first (run-in) and third (recovery) periods, 50 mls N saline was infused. During the second, either gludopa (25 μ g/kg/min) or glutyrosine (25 μ g/kg/min) was administered in 50 mls N saline in random order. Blood sampling was performed before subjects stood to void, at hourly intervals, except for two 30 min samples after the onset of dipeptide infusion. Pulse and blood pressure were measured, in duplicate, at each sampling point. For analysis, the control results were those for the second hour of the run-in period.

Results

No side-effects were reported during the infusion of either dipeptide. There were no significant differences in control values between the two days of study. During the infusion of gludopa, urine dopamine excretion increased from a control value of 0.95 ± 0.08 nmol/min to a maximum of 776.7 ± 35.0 nmol/min in the second hour of infusion (Figure 1a-1). Conversion to dopamine was still continuing at high levels at the end of the recovery period (2 hours after the infusion of gludopa had finished). Over the 4 hour period following the commencement of the infusion, conversion to urine dopamine was 18% of the dipeptide given (on a molar basis). By contrast, there was no appreciable conversion of glutyrosine to dopamine, and the levels of dopamine were not significantly elevated above baseline (Figure 1a-1).

Sodium output almost doubled in the first hour of gludopa infusion, rising

from 0.14 ± 0.03 to 0.26 ± 0.04 mmol/min ($p < 0.01$, Figure 1a-2). Output continued to rise and peaked in the hour after the gludopa infusion stopped. The natriuresis was accompanied by a diuresis, urine flow rising from 4.6 ± 1.1 to 7.6 ± 0.8 mls/min ($p < 0.05$) in the first hour of infusion. Urine potassium output also showed a small non-significant increase during the first hour (Figure 1a-3). Glutyrösine did not produce any change in sodium, potassium or volume output. Plasma renin activity fell from 1.61 ± 0.22 ng angiotensin I/ml/hr at control to 0.94 ± 0.18 ng angiotensin I/ml/hr after 30 minutes of infusion of gludopa ($p < 0.01$). PRA fell further after this point and remained depressed throughout the study. With glutyrösine, there was a non-significant rise in renin (1.04 ± 0.2 to 1.32 ± 0.50 ng angiotensin I/ml/hr at one hour (Figure 1a-4). Gludopa caused a short-lived significant rise in urine kallikrein at 30 minutes (0.50 ± 0.06 to 0.81 ± 0.17 Eu/hr, $p < 0.05$), but the levels had returned to the baseline by one hour. With glutyrösine, there was a small insignificant rise (Figure 1a-5).

There were no significant changes in blood pressure or pulse rate during infusion of either dipeptide when compared to control values, though at 2 hours mean blood pressure was significantly lower with gludopa compared to glutyrösine (Table 1a-1).

	Time (hours)				
	C	1	2	3	4
Gludopa					
Mean arterial pressure (mm Hg)	81.7 ± 4.3	80.8 ± 3.6	76.7† ± 3.4	78.8 ± 3.2	79.0 ± 2.8
Mean pulse rate (beats/min)	64.0 ± 4.0	62.0 ± 4.0	62.0 ± 4.0	61.0 ± 4.0	64.0 ± 5.0
Glutyrosine					
Mean arterial pressure (mm Hg)	83.5 ± 1.4	83.7 ± 3.0	83.0† ± 2.9	80.5 ± 3.6	81.0 ± 2.8
Mean pulse rate (beats/min)	61.0 ± 3.0	58.0 ± 3.0	61.0 ± 3.0	60.0 ± 3.0	61.0 ± 3.0

Table 1A-1 Mean blood pressures and pulse rates in six subjects in response to gludopa (25 µg/kg/min) and glutyrosine (25 µg/kg/min) infused during hours 1 and 2. Values are means ± SEM, † p < 0.05 for comparisons between gludopa and glutyrosine.

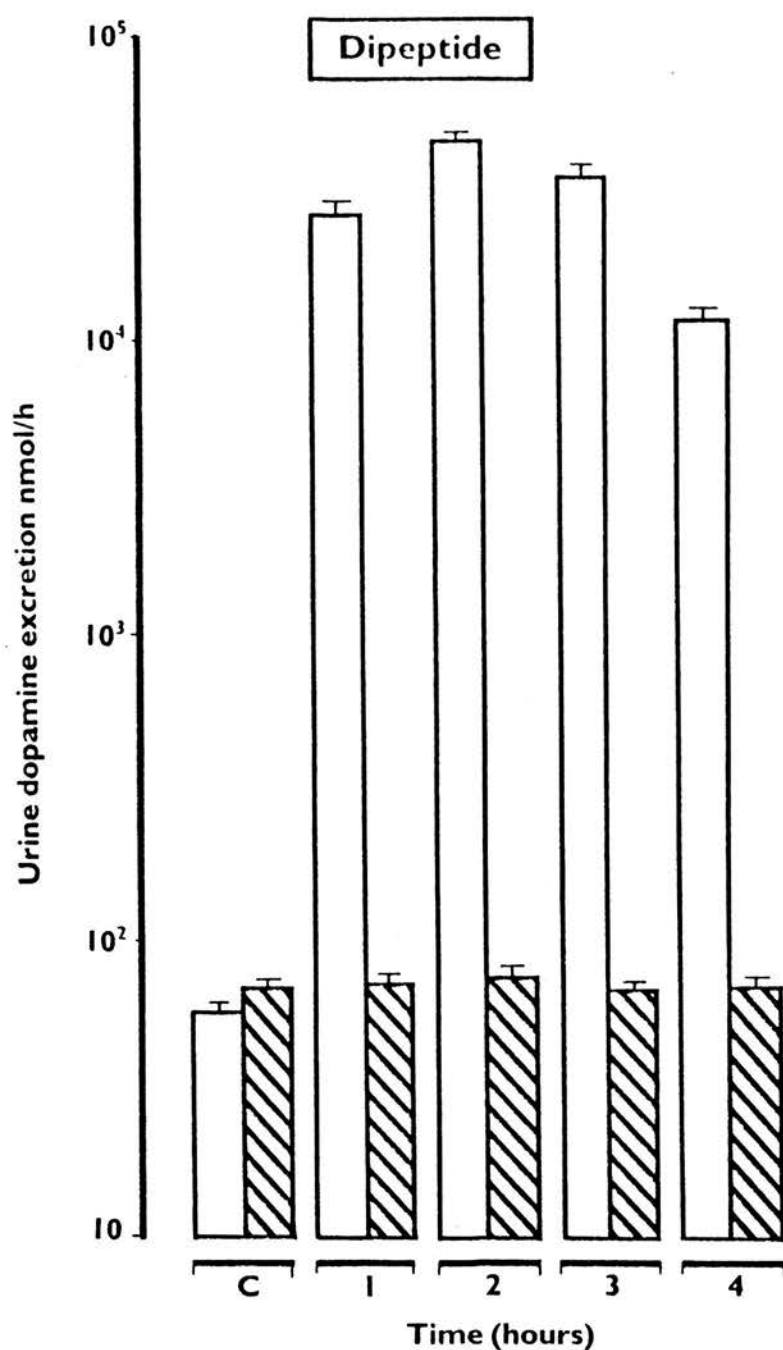


Figure 1a-1. Urine dopamine excretion in six subjects receiving either glutodopa ($25\mu\text{g/kg/min}$, \square) or glutyrosine ($25\mu\text{g/kg/min}$, \square). Values are means \pm SEM.

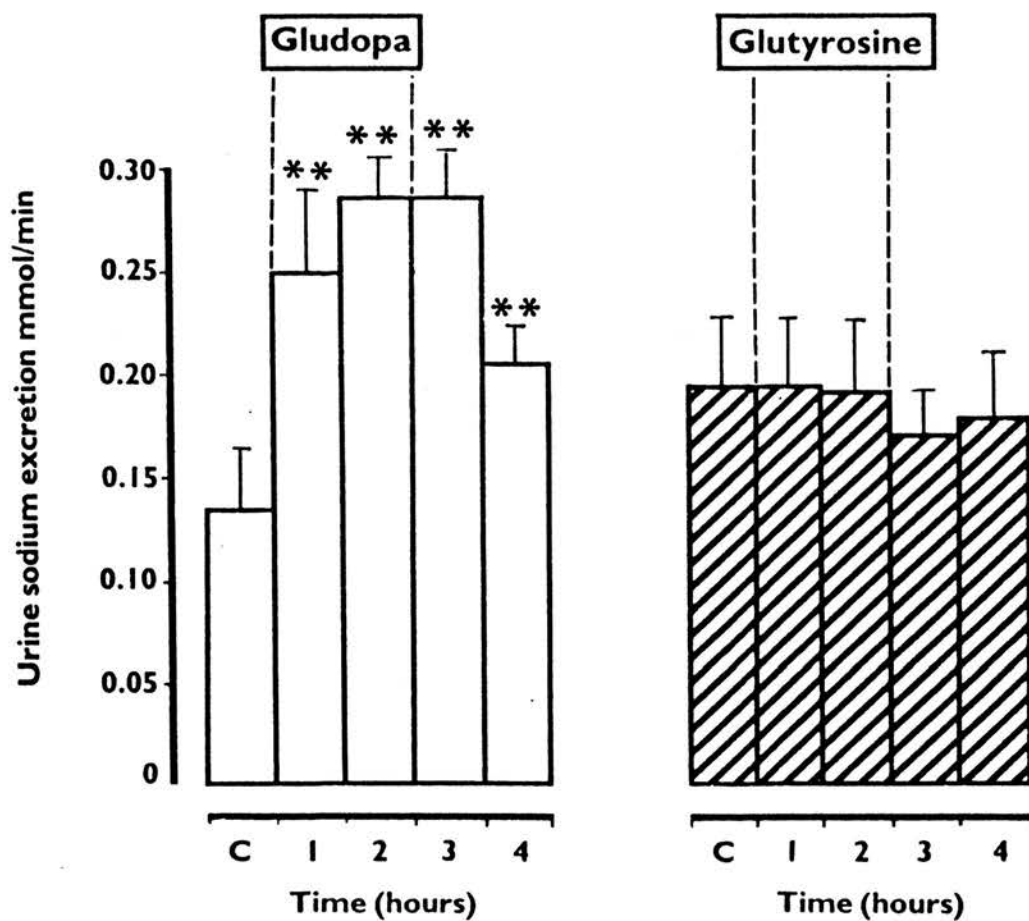


Figure 1a-2. Urine sodium excretion in six subjects receiving either gludopa ($25\mu\text{g/kg/min}$) or glutyrösine ($25\mu\text{g/kg/min}$). Values are means \pm SEM; ** $p < 0.01$, * $p < 0.05$ for comparison with control.

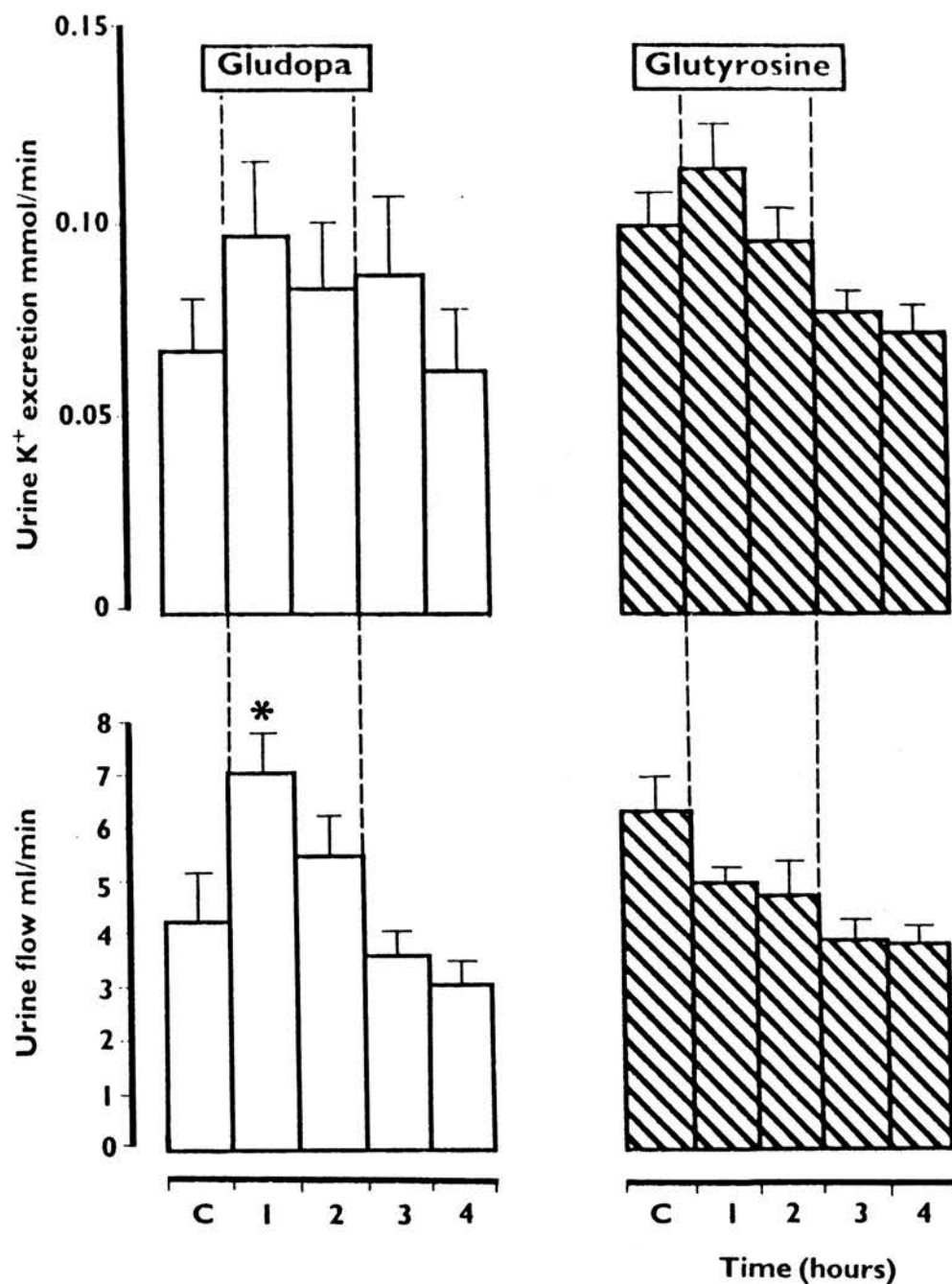


Figure 1a-3. Urine potassium excretion and flow in six subjects receiving either gludopa ($25\mu\text{g/kg/min}$) or glutytrosine ($25\mu\text{g/kg/min}$). Values are means \pm SEM; $p < 0.05$ for comparison with control.

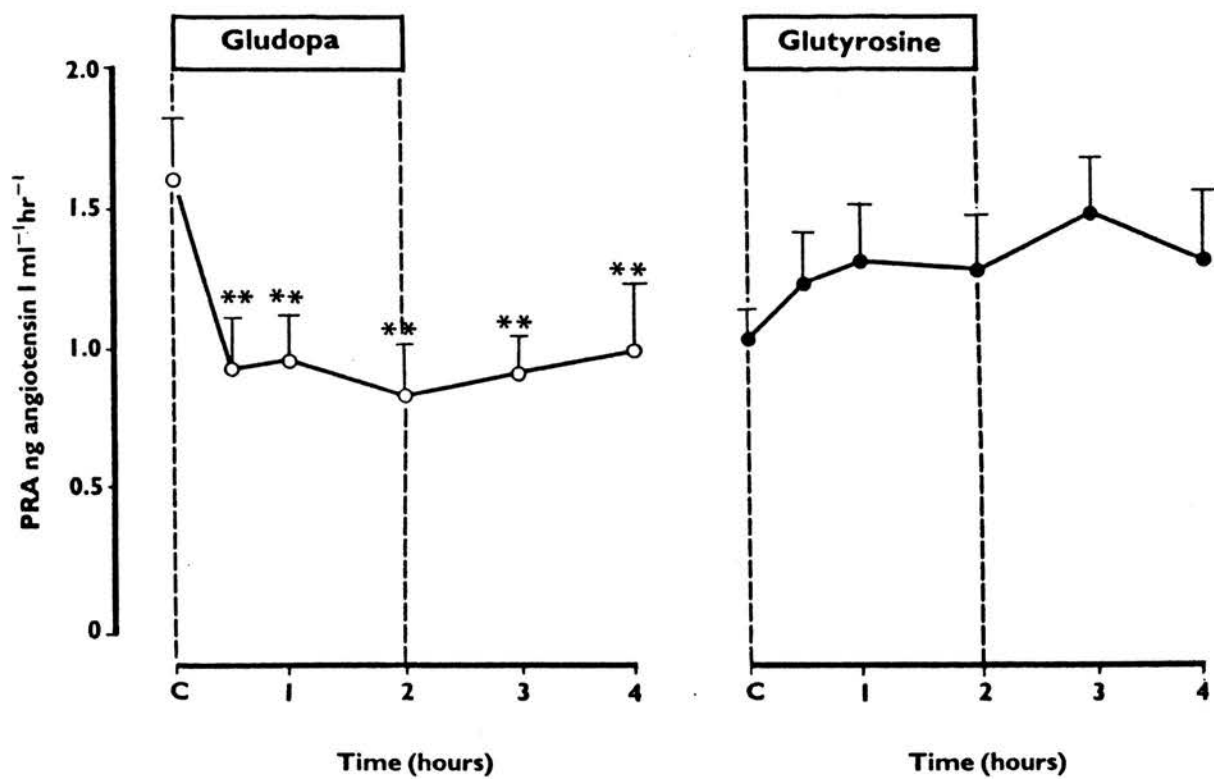


Figure 1a-4. Plasma renin activity in six subjects receiving either gludopa ($25\mu\text{g/kg/min}$) or glutyrösine ($25\mu\text{g/kg/min}$). Values are means \pm SEM; ** $p < 0.01$ for comparison with control.

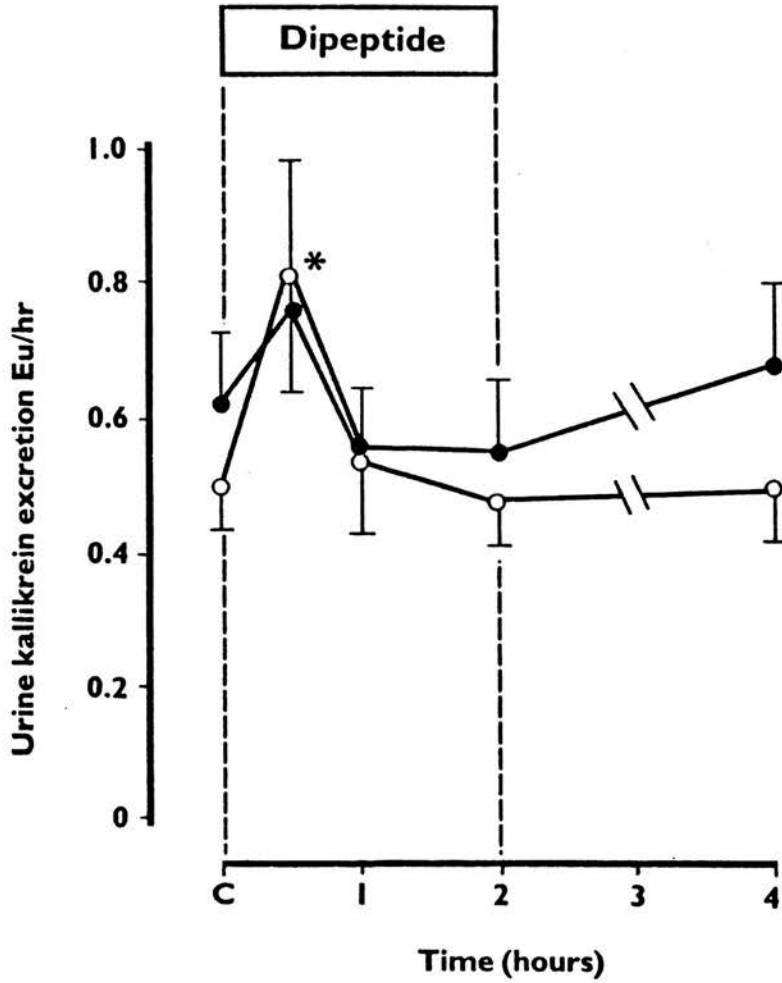


Figure 1a-5. Urine kallikrein excretion in six subjects receiving either glutodopa ($25 \mu\text{g/kg/min}$, \circ) or glutyrosine ($25 \mu\text{g/kg/min}$, \bullet). Values are means \pm SEM; * $p < 0.05$ for comparison with control

1(b) THE EFFECT OF CARBIDOPA AND INDOMETHACIN ON THE RENAL
RESPONSE TO γ -L-GLUTAMYL-L-DOPA

Protocol

Seven volunteers took part, five completing 3 days and two completing 2 days of study. Comparisons were thus made for the effect of placebo against carbidopa, and placebo against indomethacin, on the responses to gludopa. Five subjects also took part in study 1a and the results from the day of gludopa infusion were used in the present study. As in 1a, gludopa (25 μ /kg/min) was infused in 50 mls N saline during the middle 2 hour period of a 6 hour study. Placebo, indomethacin (100 mg) or carbidopa (100 mg) were given by mouth after the first hour of the run-in period. Blood pressure and pulse were measured and blood and urine samples collected in an identical manner to study 1a. For analysis, the control results were those for the second hour of the run-in period.

Results

No side-effects were reported during any part of the study.

Effect of carbidopa on responses to gludopa

There were no significant differences in the control results between the two days. Table 1b-1 shows the urine dopamine response to gludopa. Excretion increased on average nearly 900-fold by the second hour of infusion with output continuing at high levels two hours after the end of the infusion. Over 4 hours mean total dopamine excretion was 18.7% of the gludopa administered on a molar basis. However, in the presence of carbidopa, urine dopamine increased only 24-fold on average, representing a reduction in the conversion of gludopa

to dopamine by 97%. Over the four hour period, the percentage conversion to dopamine was only 0.7% of the dipeptide infused. Sodium output (Figure 1b-1) increased from 0.12 ± 0.02 to 0.34 ± 0.04 mmol/min ($p < 0.01$) during the second hour of gludopa infusion and excretion was still elevated above baseline at the end of the study period. PRA fell sharply after the onset of gludopa from 1.3 ± 0.24 to 0.82 ± 0.19 ng angiotensin I/ml/hr at 30 min ($p < 0.01$) and levels remained suppressed after the infusion (Figure 1b-2). Gludopa caused a short-lived, though significant increase in urine kallikrein but levels had returned to baseline by 1 hour (Figure 1b-3). Table 1b-3 shows that blood pressure tended to fall insignificantly with gludopa, although at 2 hours the mean value was significantly lower than that in the presence of carbidopa.

By contrast, in the presence of carbidopa, the effects of gludopa were abolished. Sodium excretion and kallikrein output did not increase above baseline, and a small fall in PRA occurred but was not significant. There were no significant changes in pulse and mean blood pressure, compared to the control period.

Effect of indomethacin on responses to gludopa

There were no significant differences in the control results between the two days.

As in the first study, gludopa significantly increased sodium excretion and lowered plasma renin activity (figures 1b-4,5). Indomethacin did not alter the conversion of gludopa to dopamine (Table 1b-2) and the total output in the 4 hours after gludopa was similar to that following gludopa alone. The natriuresis was not significantly altered by prior administration of indomethacin and the total sodium outputs were similar (61.2 ± 13.0 vs 76.4 ± 13.6 mmol, N.S.). PRA sharply declined after indomethacin, a known effect, and levels continued

to fall during and after gludopa (Figure 1b-5). In the presence of indomethacin, urine kallikrein output did not change throughout the study (Figure 1b-6). Mean blood pressure tended to be higher one hour after indomethacin and remained so throughout the study such that values were significantly elevated at two hours compared to those with gludopa alone. In the presence of indomethacin, the mean pulse rate tended to be lower during the control period and declined during the infusion of gludopa, though the changes were not significant (Table 1b-4).

	Time (hours)				
	C	1	2	3	4
Gludopa	1.02 ± 0.09	487.0 ± 42.8	880.1 ± 46.1	655.3 ± 55.8	266.1 ± 46.0
Gludopa and Carbidopa	1.22 ± 0.08	11.8 ± 1.8	24.3 ± 4.7	23.2 ± 5.8	13.8 ± 2.3

Table 1B-1 Urine dopamine output (nmol/min) in six subjects in response to gludopa (25 µg/kg/min), infused during hours 1 and 2, with or without pretreatment with carbidopa (100 mg). Values are means ± SEM.

	Time (hours)				
	C	1	2	3	4
Gludopa	1.04 ± 0.08	496.6 ± 42.7	886.3 ± 48.2	596.2 ± 43.0	212.8 ± 14.7
Gludopa and Indomethacin	1.31 ± 0.13	507.4 ± 28.5	984.1 ± 69.7	593.6 ± 47.7	194.4 ± 11.2

Table 1B-2 Urine dopamine output (nmol/min) in six subjects in response to gludopa (25 µg/kg/min) with or without pretreatment with indomethacin (100 mg). Values are means ± SEM.

	Time (hours)				
	C	1	2	3	4
Gludopa					
Mean arterial pressure (mm Hg)	86.8 ± 4.8	85.2 ± 3.8	81.0† ± 3.5	83.2 ± 4.1	83.4 ± 4.8
Mean pulse rate (beats/min)	67.0 ± 3.0	66.0 ± 2.0	67.0 ± 3.0	66.0 ± 3.0	68.0 ± 3.0
Gludopa and Carbidopa					
Mean arterial pressure (mm Hg)	88.8 ± 3.8	91.3 ± 2.8	91.0† ± 2.6	91.2 ± 3.3	94.8 ± 3.9
Mean pulse rate (beats/min)	64.0 ± 2.0	65.0 ± 2.0	62.0 ± 2.0	62.0 ± 2.0	68.0 ± 2.0

Table 1B-3 Systemic responses to gludopa (25 µg/kg/min) infused during hours 1 and 2 in six subjects, with or without pretreatment with carbidopa (100 mg). Values are means ± SEM, † p < 0.05 for comparison between gludopa alone and gludopa with carbidopa.

	Time (hours)				
	C	1	2	3	4
Gludopa					
Mean arterial pressure (mm Hg)	84.8 + 3.4	83.5 + 2.8	80.0† + 2.8	81.5 + 2.8	81.6 + 3.4
Mean pulse rate (beats/min)	67.0 + 3.0	66.0 + 2.0	67.0 + 3.0	65.0 + 3.0	70.0 + 3.0
Gludopa and Indomethacin					
Mean arterial pressure (mm Hg)	90.5 + 3.5	90.5 + 2.3	89.0† + 3.3	89.5 + 2.1	92.0 + 2.2
Mean pulse rate (beats/min)	62.0 + 4.0	58.0 + 2.0	59.0 + 3.0	61.0 + 3.0	62.0 + 3.0

Table 1B-4 Systemic responses to gludopa (25 µg/kg/min) infused during hours 1 and 2, in six subjects, with or without pretreatment with indomethacin (100 mg). Values are means + SEM, † p < 0.05 for comparison between gludopa alone and gludopa with indomethacin.

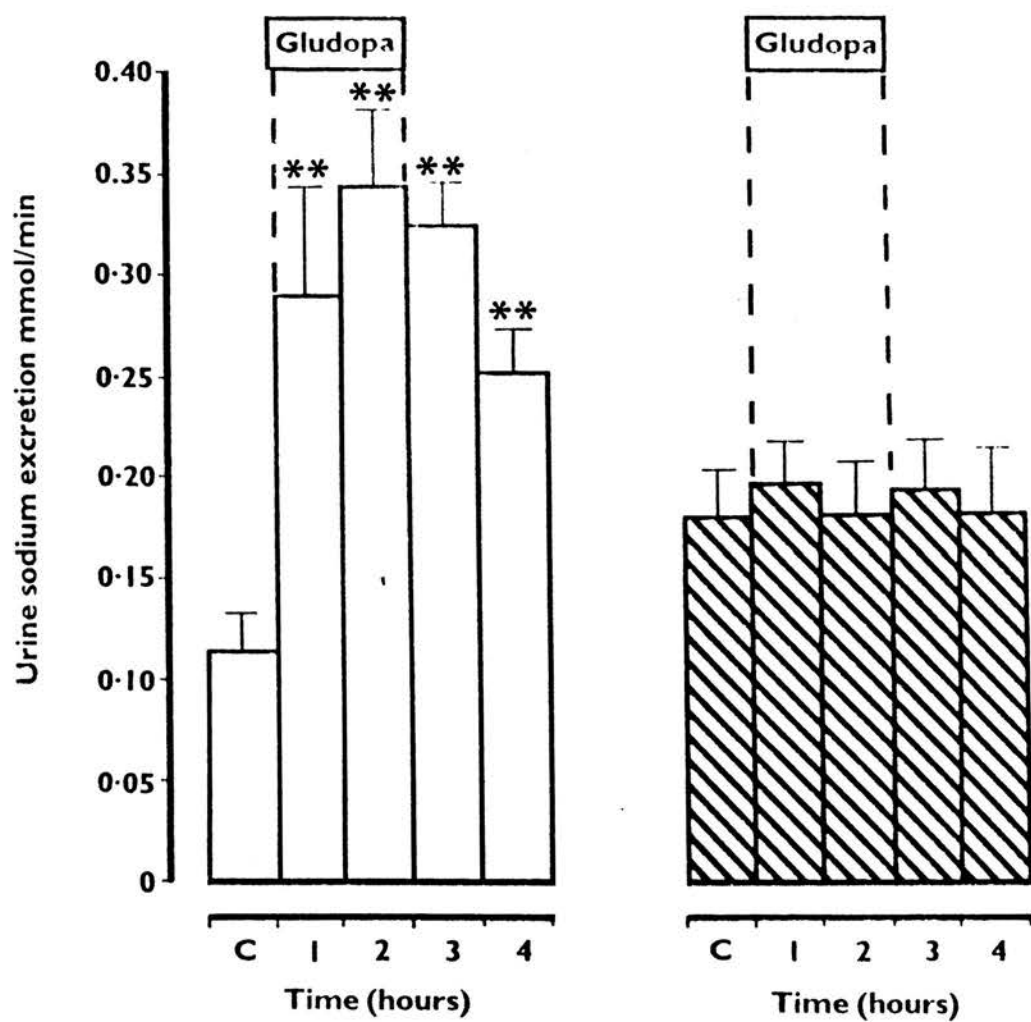


Figure 1b-1. Urine sodium excretion in six subjects receiving gludopa ($25\mu\text{g/kg/min}$) with (\blacksquare) or without (\square) pretreatment with carbidopa (100 mg). Values are means \pm SEM; ** $p < 0.01$ for comparison with control.

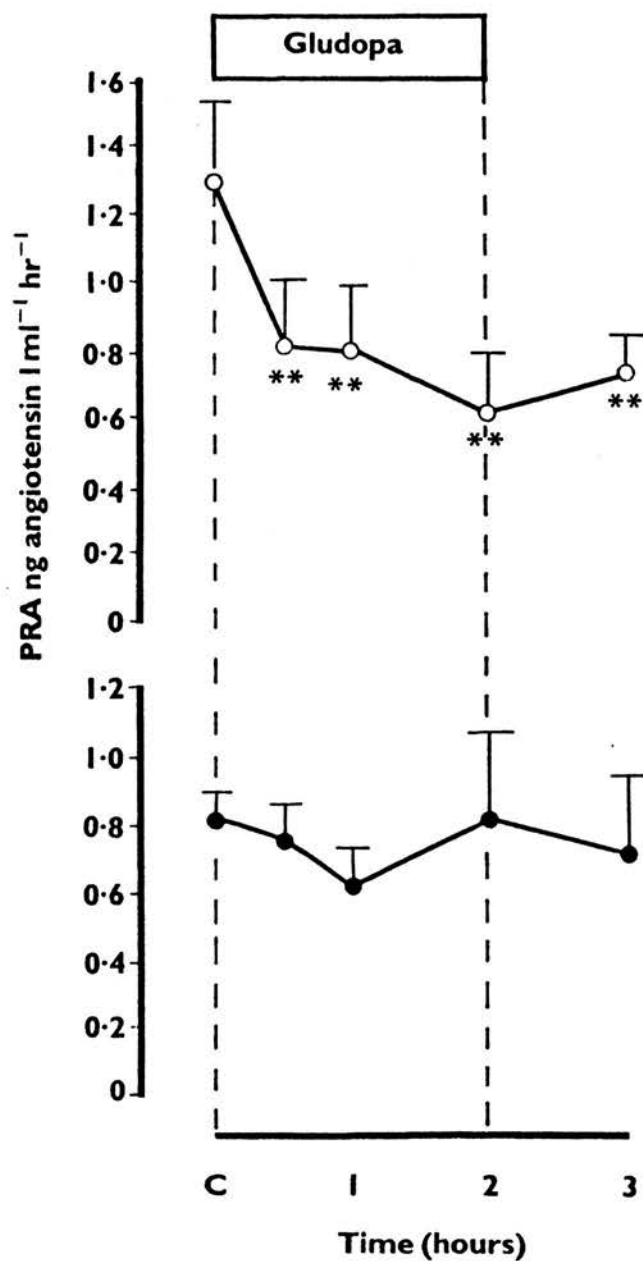


Figure 1b-2. Response of plasma renin activity to gludopa ($25\mu\text{g/kg/min}$) in six subjects with (●) or without (○) pretreatment with carbidopa (100mg). Values are means $\pm\text{SEM}$; ** $p<0.01$ for comparison with control.

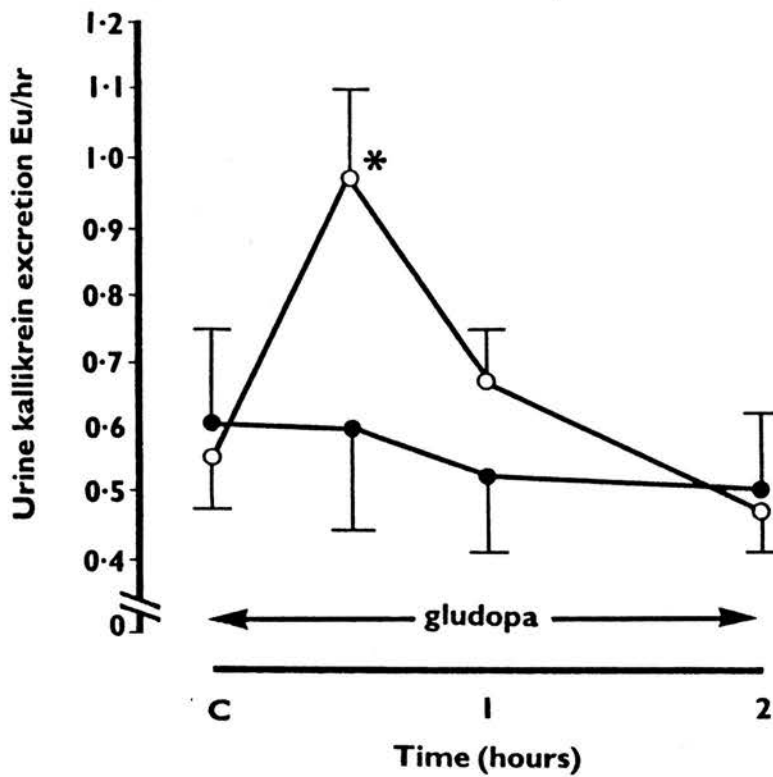


Figure Ib-3. Urine kallikrein excretion in six subjects receiving gludopa ($25\mu\text{g/kg/min}$) with (●) or without (○) pretreatment with carbidopa (100 mg). Values are means \pm SEM; * $p < 0.05$ for comparison with control.

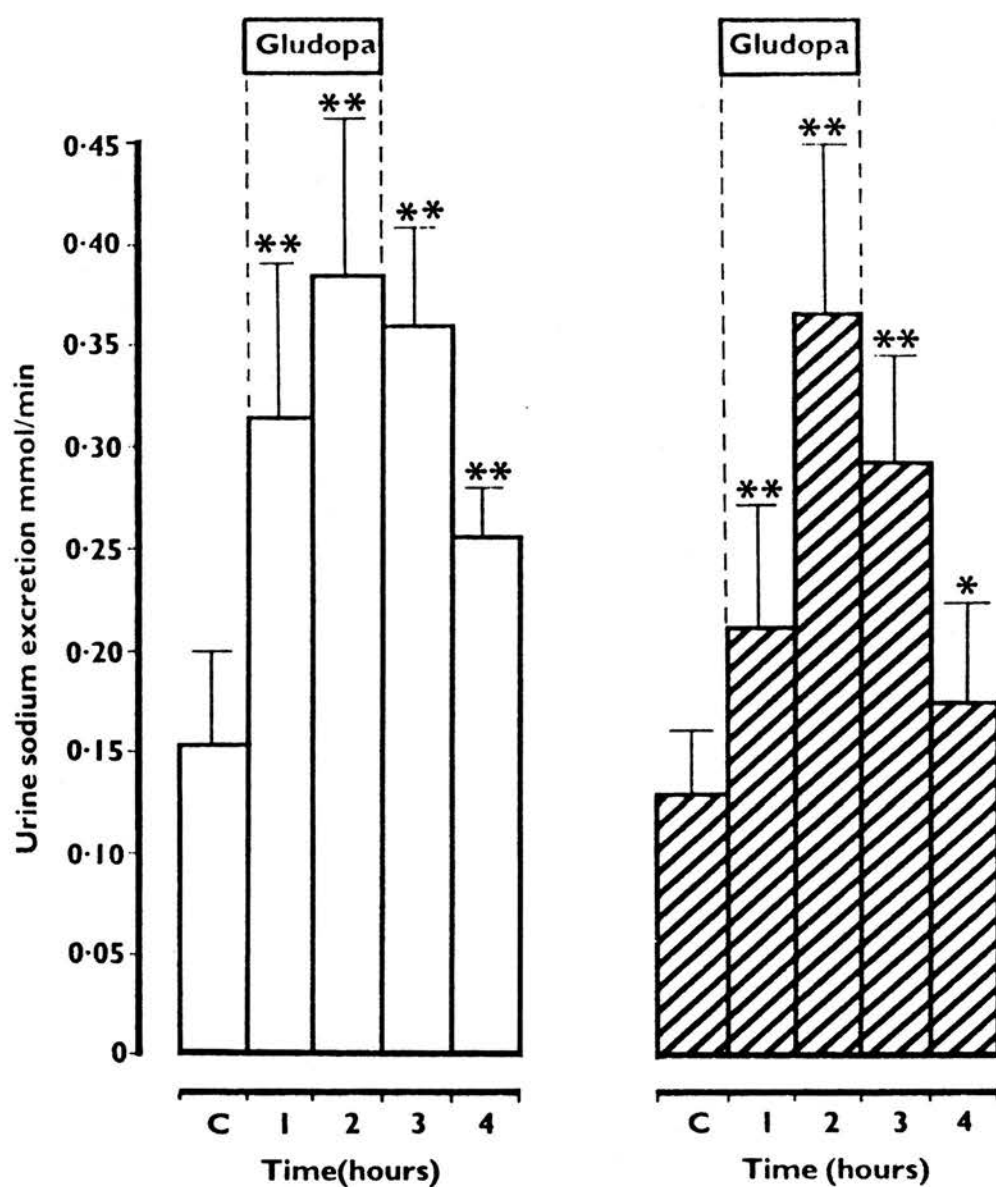


Figure 1b-4. Urine sodium excretion in six subjects receiving gludopa ($25\mu\text{g/kg/min}$) with (\square) or without (\square) pretreatment with indomethacin (100mg). Values are means \pm SEM, ** $p < 0.01$, * $p < 0.05$ for comparison with control.

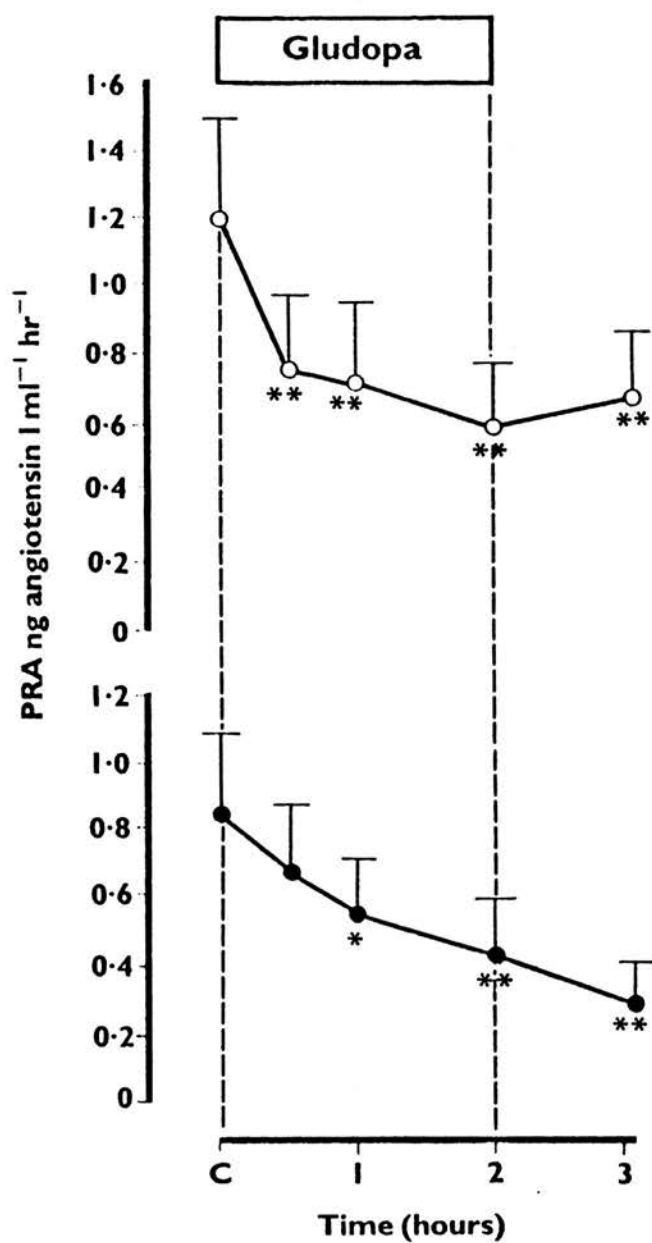


Figure Ib-5. Response of plasma renin activity to gludopa (25 μ g/kg/min) in six subjects with (●) or without (○) pretreatment with indomethacin (100mg). Values are means \pm SEM; * $p < 0.05$, ** $p < 0.01$ for comparison with control

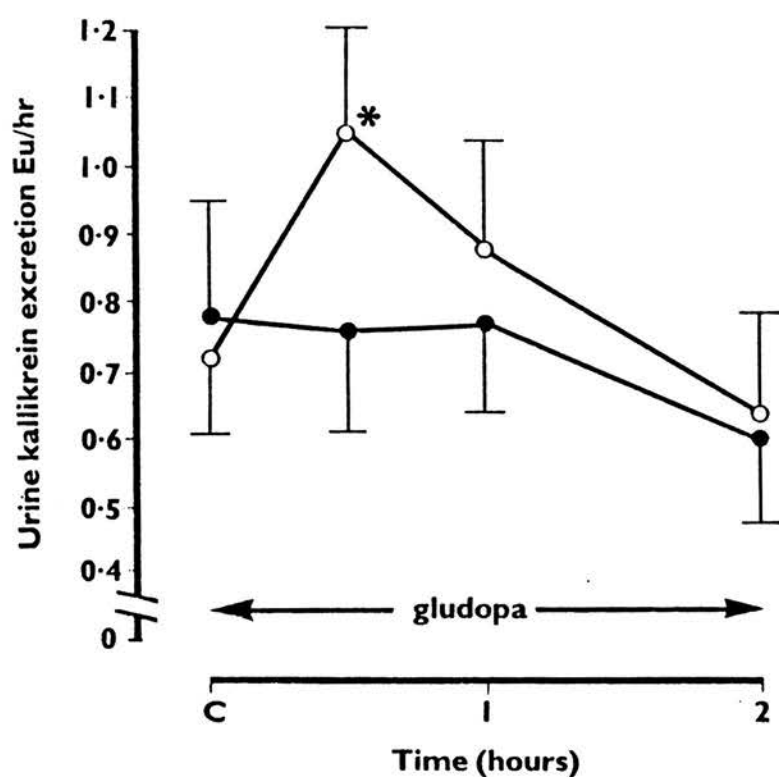


Figure Ib-6. Urine kallikrein excretion in six subjects receiving gludopa ($25\mu\text{g/kg/min}$) with (●) or without (○) pretreatment with indomethacin (100 mg). Values are means \pm SEM; * $p < 0.05$ for comparison with control.

1(c) THE EFFECT OF LITHIUM ON THE RENAL RESPONSE TO γ -L-GLUTAMYL-L-DOPA

Protocol

Seven volunteers were each studied on two occasions. Each day consisted of three 90 minute periods. During the first (run-in) and third (recovery), 50 mls N saline were infused. Gludopa (25 μ g/kg/min) was infused during the second in 50 mls N saline. On one occasion, lithium carbonate (750 mg, Camcolit) was taken at 2200 hours on the preceding evening so that subjects were studied 11 hours after administration. Blood pressure and pulse measurement, and blood and urine samples were taken at 45 minute intervals. For analysis, control results represent the calculated mean values for the two 45 minutes collections obtained during the run-in period.

Results

No subject complained of any side-effects after lithium administration or during infusion of gludopa.

Gludopa alone increased sodium output from 0.18 ± 0.03 mmol/min during the control period to 0.44 ± 0.08 mmol/min during the second 45 minutes of the infusion ($p < 0.01$, Table 1c-1). Excretion remained elevated above control at the end of the study. PRA was acutely suppressed by gludopa from 1.40 ± 0.37 to 0.85 ± 0.18 ng angiotensin I/ml/hr ($p < 0.01$) and levels remained suppressed after the infusion (Figure 1c-1).

Serum lithium concentration was 0.29 ± 0.09 mmol/l at the start of the study falling to 0.24 ± 0.09 mmol/l at the end (Figure 1c-2). As shown in Figure 1c-3, examination of sodium outputs during the control periods on the two days demonstrates that lithium alone was natriuretic (0.27 ± 0.04 vs $0.18 \pm$

0.03 mmol/min, $p < 0.01$). In the presence of lithium, however, gludopa did not produce a significant natriuresis (Table 1c-1) and the marked attenuation of response both during and after gludopa is shown in Figure 1c-4. Cumulative natriuresis above control values, during the three hours after the start of infusion, was significantly reduced (41.8 ± 9.5 to 14.5 ± 8.0 mmol, $p < 0.01$, Figure 1c-5). Pretreatment with lithium resulted in an increase in PRA during the control period (2.38 ± 0.37 vs 1.40 ± 0.37 ng angiotensin I/ml/hr, $p < 0.05$, Figure 1c-1). On infusion of gludopa however, there was a significant suppression of PRA ($p < 0.01$) of similar magnitude to that for gludopa alone (around 40%) and the curves are almost parallel. Lithium did not affect urine flow either in the control period or during infusion of gludopa (Table 1c-1).

Gludopa produced an 800-fold increase in urine dopamine excretion. It is evident from Table 1c-1 that lithium did not influence dopamine output either during the control period or after gludopa stimulation. Gludopa was both phosphaturic and uricosuric (Table 1c-1). The rise in urate excretion occurred during the infusion but levels thereafter returned to baseline. However, phosphate excretion continued to increase both during and after the infusion and levels were more than doubled at the end of the study. In the presence of lithium, a significant increase in urate excretion was not seen, although a small rise was apparent. The increase in phosphate excretion was similar in magnitude with or without lithium and there were no significant differences between the responses at any point. Blood pressure tended to fall during and after the infusion of gludopa and the reduction was significant at 1 and 3 hours, compared to control. The presence of lithium did not influence blood pressure and pulse rate (Table 1c-2). Table 1c-3 shows the results of the clearance estimations. There were no significant changes in creatinine clearance on the two days, and although lithium clearance and fractional lithium clear-

ance tended to increase, the change was small and not significant. The urinary excretion of cAMP was not altered by pretreatment with lithium, and the infusion of gludopa did not significantly alter output in comparison to the control period (Figure 1c-6). Plasma ANP was 43.4 ± 6.2 pg/ml during the control period and 44.4 ± 5.4 pg/ml after gludopa. The respective figures after lithium pretreatment were 37.0 ± 4.1 and 34.8 ± 6.2 pg/ml. None of the changes reached significance.

	Time (45 min period)				
	C	1	2	3	4
Without Lithium					
Sodium excretion (mmol/min)	0.18 ± 0.03	0.31* ± 0.05	0.44** ± 0.08	0.38** ± 0.06	0.30* ± 0.04
Urine flow (mls/min)	8.9 ± 1.0	8.4 ± 0.6	9.6 ± 0.8	4.3 ± 0.4	5.3 ± 0.6
Dopamine excretion (nmol/min)	1.12 ± 0.06	407 ± 30	854 ± 52	512 ± 53	285 ± 49
Urate excretion (µmol/min)	3.46 ± 0.44	3.61 ± 0.31	4.16** ± 0.32	3.70 ± 0.24	3.43 ± 0.26
Phosphate excretion (µmol/min)	9.2 ± 2.2	12.5 ± 2.7	17.8** ± 2.7	19.0** ± 2.0	23.0** ± 2.4
With Lithium					
Sodium excretion (mmol/min)	0.27 ± 0.04	0.36 ± 0.07	0.37 ± 0.05	0.34 ± 0.05	0.26 ± 0.04
Urine flow (mls/min)	10.0 ± 0.6	9.2 ± 1.3	8.8 ± 0.6	4.5 ± 0.6	6.0 ± 0.7
Dopamine excretion (nmol/min)	1.18 ± 0.11	435 ± 36	889 ± 68	574 ± 37	238 ± 21
Urate excretion (µmol/min)	3.57 ± 0.40	3.79 ± 0.41	3.92 ± 0.27	3.69 ± 0.35	3.39 ± 0.32
Phosphate excretion (µmol/min)	9.8 ± 1.2	12.2 ± 1.9	15.5** ± 2.4	17.0** ± 2.1	19.9** ± 2.3

Table 1C-1 Responses to gludopa (25 µg/kg/min), infused during the 1st and 2nd 45 min collections, in seven subjects with or without lithium carbonate (750 mg) pretreatment. Values are means ± SEM; * $p < 0.05$, ** $p < 0.01$ compared to control.

	Time (45 min period)				
	C	1	2	3	4
Without Lithium					
Mean arterial pressure (mm Hg)	87.7 + 2.3	81.5* + 2.2	84.5 + 2.9	81.7* + 3.1	84.5 + 4.6
Mean pulse rate (beats/min)	65.0 + 2.0	62.0 + 2.0	68.0 + 2.0	64.0 + 2.0	67.0 + 2.0
With Lithium					
Mean arterial pressure (mm Hg)	86.2 + 3.5	85.0 + 3.4	83.3 + 2.3	81.3 + 3.1	85.0 + 4.0
Mean pulse rate (beats/min)	67.0 + 4.0	66.0 + 3.0	66.0 + 2.0	67.0 + 3.0	69.0 + 3.0

Table 1C-2 Mean blood pressure and pulse rates in seven subjects in response to gludopa (25 µg/kg/min) infused during the 1st and 2nd 45 min collections, with or without lithium carbonate (750 mg) pretreatment. Values are mean ± SEM; * p < 0.05 compared to control.

	Control	Gludopa	Recovery
Without Lithium			
Creatinine clearance (mls/min)	154.5 + 7.4	159.1 + 8.8	156.0 + 8.1
With Lithium			
Creatinine clearance (mls/min)	150.4 + 9.0	155.6 + 9.4	152.2 + 12.4
Lithium clearance (mls/min)	28.4 + 4.9	30.7 + 4.5	32.7 + 6.0
Fractional lithium clearance (%)	18.9	19.7	21.5

Table 1C-3 Effect of gludopa (25 $\mu\text{g/kg/min}$) on clearance values with or without lithium carbonate (750 mg) pretreatment in seven subjects. Values are means \pm SEM.

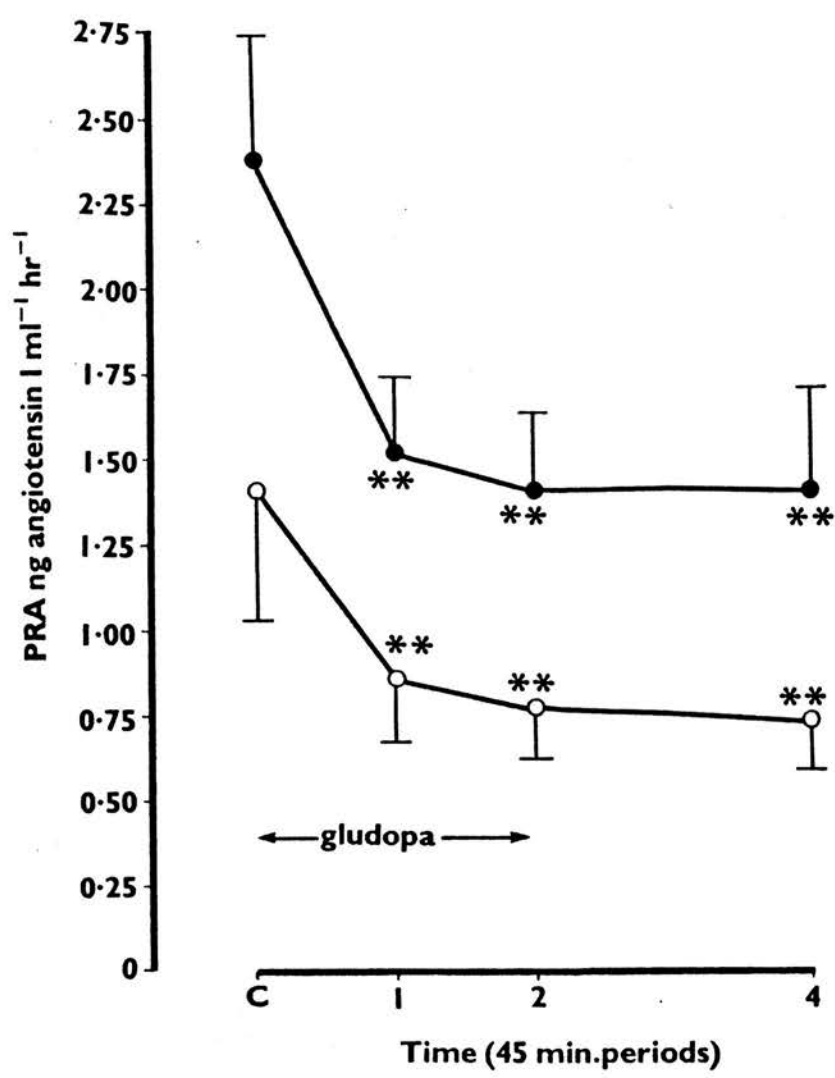


Figure 1c-1. Response of plasma renin activity to gludopa ($25\mu\text{g/kg/min}$) in seven subjects with (\bullet) or without (\circ) pretreatment with lithium carbonate (750mg). Values are means \pm SEM; ** $p < 0.01$, * $p < 0.05$ for comparison with control.

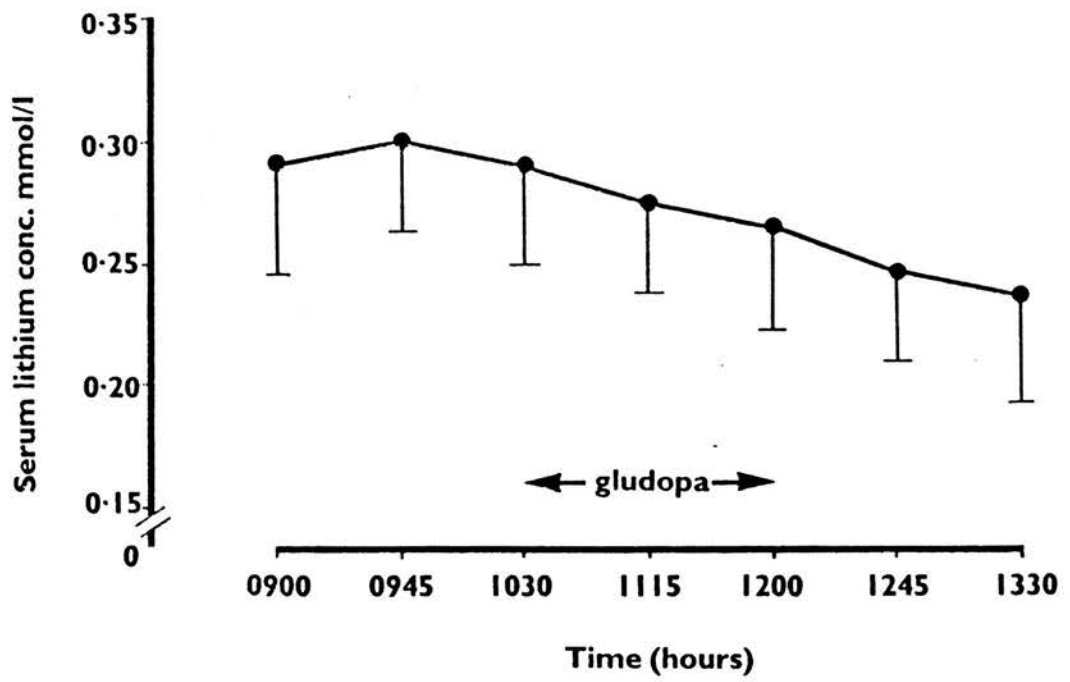


Figure 1c-2. Decline of serum lithium concentration over time of study. Values are means \pm SEM; n=7.

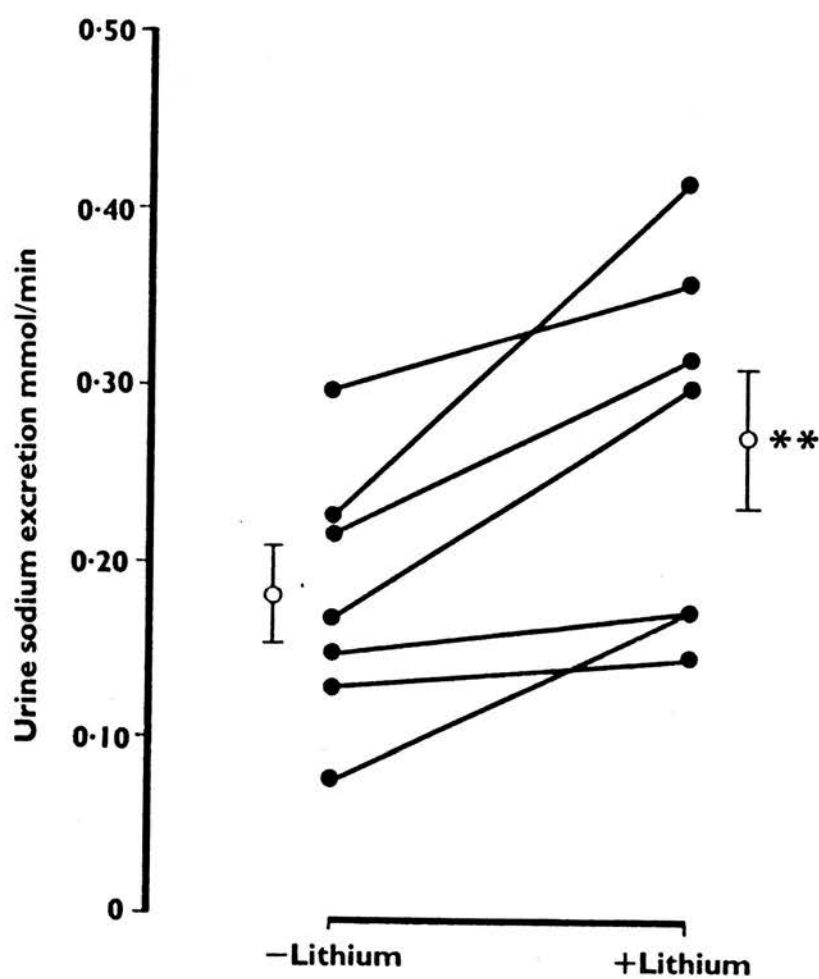


Figure 1c-3. Sodium excretion during control periods with or without pretreatment with lithium carbonate (750mg). Individual values are plotted with means \pm SEM; ** $p < 0.01$.

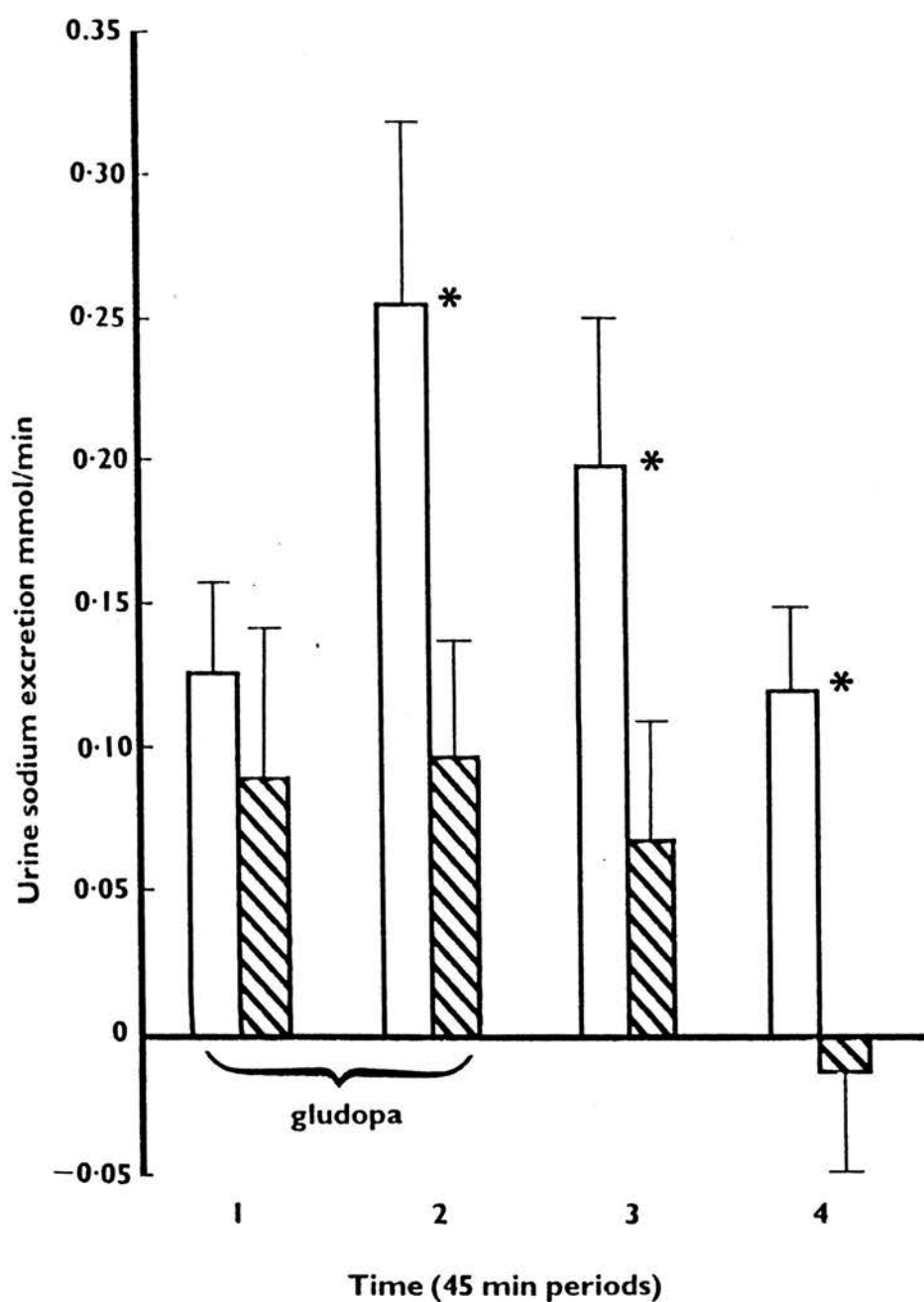


Figure 1c-4. Absolute increment of sodium excretion above control at each period during and after infusion of gludopa ($25\mu\text{g/kg/min}$), with (▨) or without (□) pretreatment with lithium carbonate (750mg). Values are means \pm SEM; $n=7$; * $p<0.05$ between different treatments.

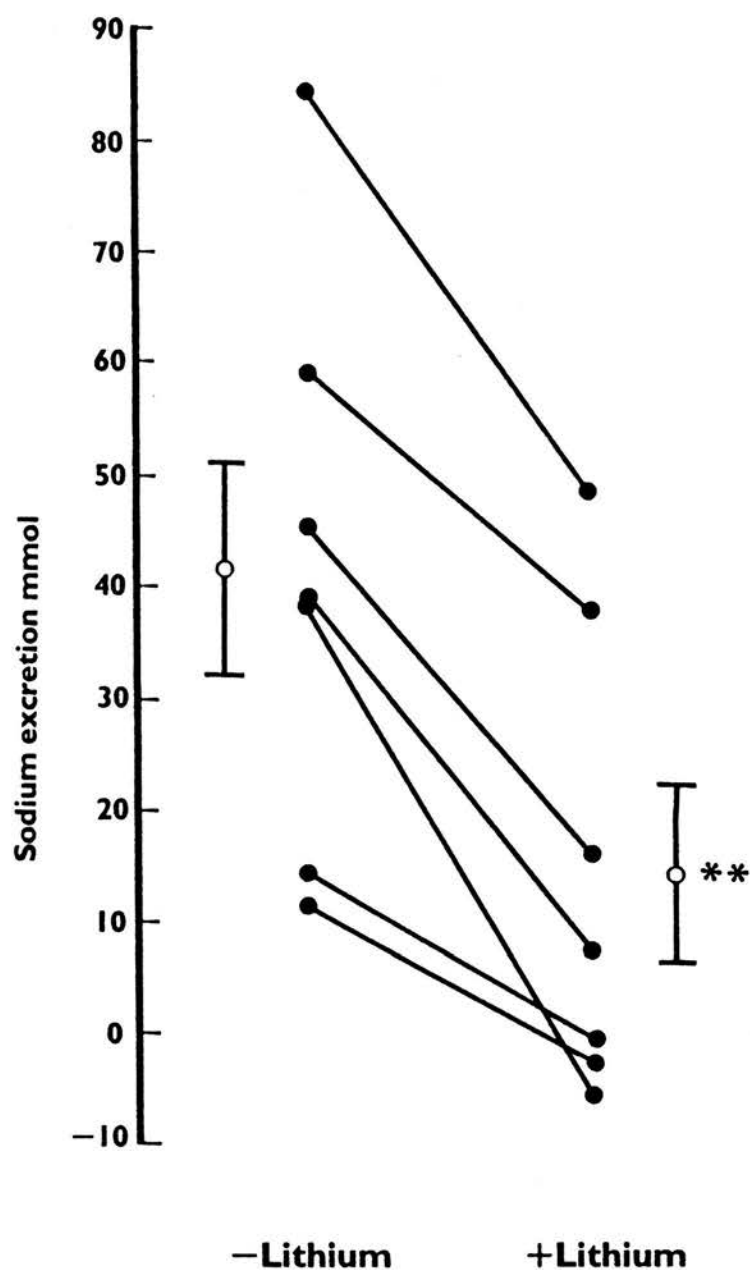


Figure Ic-5. Cumulative sodium excretion above control during and after infusion of gludopa ($25\mu\text{g/kg/min}$), with or without pretreatment with lithium carbonate (750mg). Individual values are plotted with means $\pm\text{SEM}$; ** $p < 0.01$.

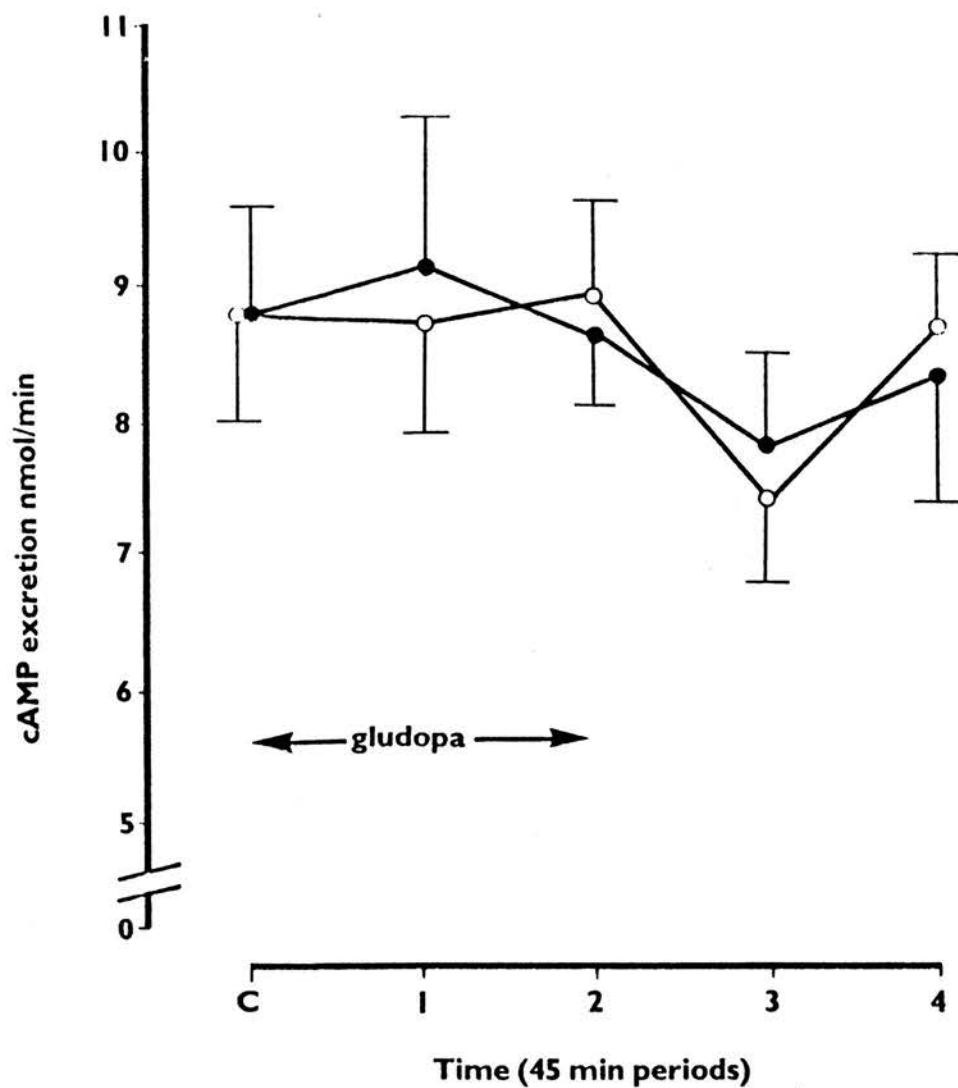


Figure 1c-6. Cyclic AMP response to gludopa ($25\mu\text{g/kg/min}$) with (●) or without (○) pretreatment with lithium carbonate (750mg) in seven subjects. Values are means \pm SEM.

DISCUSSION

Dopamine synthesis after gludopa

The studies in normal volunteers with infusion of gludopa at 25 $\mu\text{g/kg/min}$ demonstrate it to be a potent renal dopamine prodrug. Urinary dopamine output increased up to 900-fold during infusion and continued appreciably after the infusion. The results are comparable to those reported by Worth and colleagues (1985a) who infused gludopa at 12.5 and 100 $\mu\text{g/kg/min}$ and noted increases in urine dopamine of 280- and 2500-fold respectively. By comparison, infusion of dopamine within the 'renal range' at 3 $\mu\text{g/kg/min}$ produced only a 60-fold increase in urine output (Levinson et al., 1985).

During the infusion of gludopa, and the recovery period (3-4 hours in total depending on study), the excretion of dopamine accounted for around 20% of the gludopa infused, on a molar basis. As described, the relative renal specificity relies on the transformation of gludopa to dopamine under the sequential actions of γ -glutamyl transferase and dopa decarboxylase, both enzymes concentrated at the proximal tubule (Albert et al., 1961; Goldstein et al., 1972). Gludopa, with a molecular weight of 344 will be freely filtered and be available for uptake and hydrolysis by γ -glutamyl transferase at the brush border (Abbs and Kenny, 1983). In addition, enzyme on the microvasculature, in close association with the antiluminal surface of the proximal tubular cells will be exposed to large amounts of circulating substrate, the degree depending on the filtration fraction (Welbourne and Dass, 1982). The proximal tubule evidently has enormous capacity to decarboxylate L-dopa formed after deglutamation of gludopa, suggesting that dopa decarboxylase activity is not the rate limiting step in dopamine synthesis under normal conditions.

The fate of the major part of the infused gludopa cannot be entirely

answered from these studies. The fraction converted to dopamine is likely to be underestimated. In the study reported by Worth et al. (1985a) urine collections were extended and appreciable dopamine excretion was occurring at 12 hours after the infusion of gludopa. Dopamine formed in the kidney will be metabolised along a number of pathways. Some may be taken up by sympathetic nerves and form substrate for dopamine β hydroxylase, believed to be principally an intraneuronal enzyme (Nagatsu et al., 1969). Levinson and colleagues (1985) showed however, that the infusion of dopamine at 3 $\mu\text{g/kg/min}$ resulted in only a doubling of urinary noradrenaline excretion. Monoamine oxidase and catechol-O-methyl transferase are abundant within the kidney (Nagatsu et al., 1969), and the former at least is concentrated at the proximal tubule (Denney et al., 1983). The metabolic products of these enzymes, 3-hydroxytyramine, homovanillic acid and 3,4, dihydroxyphenylacetic acid (DOPAC) are likely to form an unknown proportion of the total dopamine synthesised. Under normal conditions, some 80% of urine dopamine is in the conjugated form principally the 3-O-sulphate and glucuronide (Kuchel et al., 1979). After the infusion of gludopa, work from this department (unpublished) has indicated a 20-fold increase in conjugate excretion. The preponderance of dopamine conjugates in the baseline state was reversed so that the free:conjugate dopamine ratio was 44:1. It is of note that the peak in conjugate excretion was later than that for free dopamine and this in itself provides an argument against the hypothesis that free dopamine in urine is derived from the deconjugation of circulating conjugated dopamine (Unger et al., 1978).

It is possible that organs other than the kidney metabolised gludopa appreciably. From the work of Wilk and colleagues, this seems unlikely. In the rat, activity of γ -glutamyl transferase in other tissues is minor compared to the kidney (Orlowski and Szewczuk, 1961), and after the administration of

gludopa, dopamine was not appreciably accumulated by other tissues (Wilk et al., 1978). Plasma dopamine was not measured in these experiments, but the infusion of gludopa at 12.5 and 100 $\mu\text{g/kg/min}$ (Worth et al., 1985a) elevated levels only 4- and 25-fold, such results also suggesting that dopamine synthesis is not occurring to a high degree elsewhere in the body.

Previous studies have indicated that the peripheral cardiovascular effects of L-dopa are attenuated in the presence of an inhibitor of dopa decarboxylase (Watanabe et al., 1971) and this knowledge has been utilised in the treatment of Parkinson's disease, where the concurrent administration of a peripheral decarboxylase inhibitor prevents postural hypotension produced by L-dopa (Pinder et al., 1976). The present work with carbidopa (MK-486, α -methyl-L-dopa-hydrazine) demonstrates that gludopa is itself inactive and requires decarboxylation to dopamine for renal effects to be exerted. Carbidopa reduced the dopamine response to gludopa by around 97% and rendered gludopa inactive in terms of natriuresis and effect on plasma renin activity. The decarboxylase enzyme is not specific for L-dopa and catalyses the conversion of 5-hydroxytryptophan to serotonin (Hagage and Richet, 1985). However, substrate specificity is many times greater for L-dopa than 5-hydroxytryptophan in various tissue models (Sourkes, 1966; Srinivasan and Awapara, 1978). The enzyme is properly called aromatic L-amino acid decarboxylase, though such terminology has been questioned as it has little activity against other L-amino acids (Sourkes, 1966; Srinivasan and Awapara, 1978). Serotonin can, in various experimental models, be demonstrated to exert diverse pharmacological effects in the cardiovascular system. However, whether it has a physiological role in renal function is unknown. An effect on the renal synthesis and excretion of noradrenaline and adrenaline by carbidopa is disputed and has been referred to earlier. A significant reduction of these

catecholamines in the kidney would have produced opposing effects on sodium excretion and plasma renin activity to those observed. These points cannot be definitely answered, but it is clear that blockade of the renal effects of gludopa occurred simultaneously with the inhibition of dopamine synthesis and the evidence suggests that the two events are causally linked.

Peripheral nerves actively take up tyrosine, and the formation of L-dopa, under the action of tyrosine hydroxylase, is the rate limiting step in neuronal catecholamine synthesis (Nagatsu, 1973; Weiner, 1979). Nagatsu and colleagues (1969) demonstrated in the denervated dog kidney that tyrosine hydroxylase is an exclusively intraneuronal enzyme, whereas denervation did not significantly alter the activity of dopa decarboxylase. The completeness of renal denervation was confirmed by the disappearance of renal tissue noradrenaline. Baines and Chan (1980) have utilised a micropuncture technique in rats to determine requirements for renal dopamine synthesis. Injection of L-dopa into proximal tubules of denervated kidneys resulted in active dopamine formation: the similar injection of L-tyrosine did not produce a measureable response. Again, in the isolated perfused rat kidney preparation, whereas the infusion of L-dopa resulted in a large increase in dopamine output, L-tyrosine was without effect (Adam and Adams, 1985). It is known that tyrosine is actively and efficiently reabsorbed in the proximal tubule after filtration (Chan, 1976) so that in both studies, tyrosine would have been available in tubular cells for onward synthesis of dopamine: the lack of conversion must reflect the absence of tyrosine hydroxylase in proximal tubular cells, supporting the work of Nagatsu's group. The present studies further confirm these reported findings. Glutyrine, infused at pharmacological doses, would, in an analogous manner to gludopa, have produced large amounts of free amino-acid at the proximal tubule. However, urine dopamine excretion did not rise above baseline

values throughout the study, and the dipeptide did not exert effects on measured indices of renal function. Under normal conditions, L-tyrosine levels in plasma are at least 100 times those of L-dopa (Hilton, 1982; Shum et al., 1982). Thus in spite of a ready supply in the physiological state and massive input under the conditions of the present study, the kidney seems unable to utilise L-tyrosine as a source for urine dopamine. The results support the evidence that circulating L-dopa is the main physiological source for the generation of urine dopamine. The putative renal dopaminergic nerves, whilst not materially contributing to urine dopamine, may, through local action, modulate intrarenal blood flow and sodium excretion. The present study does not provide direct evidence for or against this hypothesis. However, with the availability in the kidney of large amounts of released tyrosine after infusion of glutyrosine, active uptake and increased renal dopaminergic nerve activity might have been expected. Renal blood flow was not measured, but the lack of change in sodium excretion and renin activity militates against a significant role for the dopaminergic nerves.

Dopamine, infused at doses between 1 and 4 $\mu\text{g/kg/min}$ may, by producing systemic and renal vasodilation, result in a small decrease in blood pressure (Goldberg and Murphy, 1987), though such a finding is by no means universal (McDonald et al., 1964; Connell et al., 1983; Levinson et al., 1985). In the present work, there was a tendency for blood pressure to decline during the infusion of gludopa. It is not easy to explain such findings. Worth et al. (1985a) demonstrated that gludopa, infused at 12.5 and 100 $\mu\text{g/kg/min}$ elevated plasma levels of dopamine by 4 and 25-fold respectively. The increase in plasma dopamine produced by the infusion of dopamine at between 1 and 4 $\mu\text{g/kg/min}$ varies from 300–1900 fold (Connell et al., 1983; Holland et al., 1983; Levinson et al., 1985). To achieve levels comparable to those produced by gludopa,

requires the infusion of just 0.1 $\mu\text{g}/\text{kg}/\text{min}$ of dopamine, a dose that is without renal or systemic effects. Explanation of this seeming discrepancy requires speculation on a number of possibilities. It may be that renal vasodilation per se accounts for sufficient reduction in total systemic resistance to lower blood pressure, as 20% of the cardiac output is normally to the kidneys. It is possible that venous levels of dopamine do not reflect those achieved at the level of resistance vessels due to metabolism. In this regard, there is no information as to whether dopamine can be locally generated from gludopa at the level of the vascular walls, given the ubiquitous distribution of γ -glutamyl transferase and dopa decarboxylase, albeit in small amounts. Finally, as will be discussed, renin was acutely suppressed by gludopa: reduction of circulating angiotensin II could therefore have accounted for the reduction in blood pressure through systemic vasodilation. In the present work, a placebo control day was not included, and it is conceivable that blood pressure and pulse changes represented a response to prolonged rest or diurnal rhythm. However, readings were compared to baseline values after subjects had already been supine for 1½ to 2 hours, and the steadiness of systemic parameters during infusion of glutyrosine, which appears inactive, and on into the recovery period, argues against those factors accounting for the effects seen.

Similar arguments apply to the use of L-dopa, hypotension being a common adverse effect in patients treated for Parkinson's Disease (Pinder et al., 1976). Goldberg and Murphy (1987) have stated that L-dopa, after oral administration of 1-1.5 grams, produces positive inotropic effects and increments in renal blood flow equivalent to the infusion of dopamine at 2 - 4 $\mu\text{g}/\text{kg}/\text{min}$. Rajfer et al. (1984) gave L-dopa (1.5 - 2.0 g) to heart failure patients and reported an increase in cardiac output and decline in systemic vascular resistance. However, plasma dopamine levels increased only 17-fold.

L-dopa freely crosses the blood brain barrier and central mechanisms have been postulated for its cardiovascular effects. This is unlikely to apply in the present studies as systemic dopamine does not readily enter the brain and the studies of Wilk and colleagues (1978) suggest that accumulation in the brain after gludopa is minimal.

The effects of gludopa on blood pressure and pulse are important to define. If a pro-drug such as gludopa is to attain a therapeutic role, an optimal dose must balance effective natriuresis and renin suppression against a tendency to induce systemic effects. The present dose of gludopa, 25 $\mu\text{g/kg/min}$, may be reaching the upper limit of the renally-selective range.

Natriuresis and proximal tubular function

The possible mechanisms by which dopamine enhances sodium excretion have been referred to, including the limitations of knowledge at the present time.

Gludopa, infused at 25 $\mu\text{g/kg/min}$, was actively natriuretic in the present studies, with sodium output increasing between 200 and 300% above control levels. The increase in excretion paralleled that of dopamine output in the urine, both persisting beyond the termination of the infusion.

Renal haemodynamics were not measured, but recent work in this department has indicated that gludopa, infused at the same dose, results in significant increases in renal blood flow, with lesser increases in glomerular filtration rate and a reduction in filtration fraction (MacDonald et al., 1987). Such changes alone would be expected to reduce proximal tubular sodium reabsorption by effect on peritubular physical forces. The rise in circulating dopamine after gludopa is too small to account for the effect on the renal vasculature, and a local effect must be postulated. Dopamine can be reabsorbed from the

distal tubule (Baines et al., 1979) and it is conceivable, though speculative, that dopamine, produced in the proximal tubule and released into the lumen, gains access to afferent and efferent arterioles after uptake in the region of the macula densa. Another contributing factor may be the inhibition of resting tone, particularly at the efferent arteriole, consequent to inhibition of renin release and suppression of angiotensin II (*vide infra*).

A possible direct proximal tubular effect of dopamine has been investigated by a number of workers and the evidence reviewed. In the present studies, further evidence was sought by analysing the renal excretion of phosphate and urate in response to *gludopa*. Phosphate is actively reabsorbed at the proximal tubule and Puschett et al. (1972) have shown that reabsorption is linked to that of sodium under various experimental conditions including isotonic saline diuresis and aortic constriction. Urate is freely filtered and bidirectional transport occurs in the proximal tubule, though overall over 90% is reabsorbed at this site. In both human and rat, volume expansion enhances the fractional excretion of urate (Diamond and Meisel, 1975). The observation that *gludopa* increased the urinary excretion of both anions, provides suggestive evidence for a proximal tubular site of action for dopamine. The results for phosphate excretion confirm those reported by Cuhe et al. (1976), who differentiated the natriuretic and phosphaturic effects of dopamine and L-dopa, infused into the canine renal artery. In these studies, phosphate excretion was still increasing at the end of the recovery period, at a time when the natriuretic response was declining. The explanation is unknown but may reflect separate transport mechanisms at the proximal tubule.

Studies involving the infusion of dopamine into man and animals are in general agreement that the natriuresis and renal vasodilation are mediated through stimulation of DA_1 receptors. Such receptors have been defined both

on the renal vasculature and on proximal tubular cells. Thus in dogs, the highly specific DA₁ antagonist, SCH 23390, blocked the renal effects of dopamine (Frederickson et al., 1985). Confirmatory evidence is also available after the infusion of gludopa in man. In this department, MacDonald et al. (1987) have shown that the natriuretic and renal vasodilator actions of gludopa are markedly attenuated by (+)-sulpiride, the most specific DA₁ antagonist available for use in man. However, similar actions of gludopa were unaltered by the peripheral DA₂ antagonist, domperidone (Worth et al., 1986).

Vlachoyannis et al. (1976) demonstrated an increase in urinary cAMP excretion after the infusion of dopamine into man. In the present studies, an increase in cAMP in response to gludopa was not demonstrated. The interpretation of measured cAMP levels in urine is complicated. About 60% of the urine output represents that filtered from circulating plasma (Sutherland, 1970). Of the nephrogenous component, parathyroid hormone is believed to be the principle determinant of normal levels (Broadus, 1981). Thus, that proportion of the total under the influence of dopamine may be small, and changes induced by dopamine may be masked by background levels. In addition, it is not clear to what extent levels in the urine represent local cellular events. cAMP may leave the cell preferentially across the basolateral membrane rather than into tubular lumen, and the degree of phosphodiesterase activity cannot be determined in whole animal clearance studies. Thus, although it is generally accepted that the actions of vasopressin on the distal tubule are mediated through cAMP, infusion of vasopressin does not result in an increase in the urinary excretion of the nucleotide (Sutherland, 1970). Also in a recent study, infusion of L-dopa into rats did not elevate urine cAMP excretion, but renal tissue cAMP content showed a 300% increase (Sato et al., 1987).

The renal responses to gludopa in the presence of carbidopa are of

particular interest. Carbidopa reduced the dopamine response by 97% and abolished the natriuresis. However, this still represented, on average, a 24-fold increase in urine dopamine output. It is difficult to explain why such a rise was not associated with a natriuresis. A reduction in proximal reabsorption of sodium would have been expected by direct tubular effect. However, the distal tubule has a large capacity to maintain glomerulotubular balance and will tend to minimise the effect of increases in sodium delivery from the proximal tubule. It may be that after gludopa alone, the delivery is of such a degree as to overcome distal compensation. In an early micropuncture study, Davis et al. (1968) postulated a distal tubular action for dopamine. A possible mechanism is a redistribution of renal blood flow to the inner cortex as described by Hardaker and Wechsler (1973). By increasing flow to inner portions of the kidney, dilution of the interstitium, by a "wash-out" phenomenon, would reduce passive sodium reabsorption in the ascending limb of the loop of Henlé, and disrupt distal compensatory reabsorption. It may be that the moderate increase in dopamine levels produced in the presence of carbidopa was too small to influence renal blood flow thus allowing effective distal compensation to occur.

There was an early rise in potassium excretion after gludopa, with levels thereafter returning towards control. Some studies involving the infusion of dopamine have demonstrated a kaliuresis (Vlachoyannis et al., 1976), but this is by no means a universal finding (McDonald et al., 1964; Levinson et al., 1985), and few workers have specifically addressed the issue. Proximally acting agents such as osmotic diuretics, produce increased potassium excretion both by reducing proximal reabsorption with sodium, and by increasing secretion in exchange for sodium at the distal tubule (Koushanpour and Kriz, 1986). A similar sequence would be expected with dopamine. In the present studies with

gludopa, inhibition of the renin-angiotensin system and hence removal of the principle stimulus to aldosterone, would tend to promote potassium retention, and offset an early direct kaliuretic response of dopamine. An analagous situation has been postulated for atrial natriuretic peptide, the infusion of which does not consistently increase potassium excretion (Needleman and Greenwald, 1986).

Under conditions of experimentally produced maximal water diuresis, urine flow is directly proportional to the proximal tubular natriuretic response induced by an agent. In the present studies, water was given to maintain reasonable flow rates with the principle aim of ensuring complete voiding at collection points, but no attempt was made to fully suppress vasopressin activity. Gludopa produced an early significant rise in urine flow in the first study but the rise was not sustained and did not parallel natriuresis. Evidence that dopamine may interact with vasopressin at the collecting ducts further complicates the picture and further studies will be required with protocols specifically designed to address this problem.

Renin release

The controversy as to the effect of dopamine on renin release has already been referred to. What is clear from the present studies is that PRA is consistently inhibited by gludopa at an infusion rate of 25 $\mu\text{g/kg/min}$. The results are also notable for the demonstration that inhibition occurs early, within 30 minutes of the start of infusion and levels continue to be suppressed beyond the infusion in spite of appreciable cumulative net sodium deficit. The results are in agreement with the work of Worth et al. (1985a, 1986) who demonstrated a fall in plasma renin activity with gludopa infused at 12.5 and 100 $\mu\text{g/kg/min}$.

What is unclear, is the mechanism of inhibition of renin release, given the multiple potential inputs to the juxtaglomerular cells. Renal vasodilation at relatively constant perfusion pressure would be expected to inhibit renin release, through the renal baroreceptor, as would the natriuresis per se, acting through the macula densa mechanism. However, these stimuli are unlikely to be of over-riding importance. For instance, dopamine infusion produces a marked natriuretic response and renal vasodilation, but most studies have pointed to either no effect, or stimulation of renin release (Otsuka et al., 1970; Levinson et al., 1985). In addition, in man, in the presence of domperidone, gludopa failed to influence renin in spite of renal vasodilation and natriuresis (Worth et al., 1986, *vide infra*).

In vitro and in vivo studies in animals (Imbs et al., 1975; Dzau et al., 1978; Williams et al., 1986) have provided evidence for direct dopaminergic input to the juxtaglomerular cells. Being modified myoepithelial cells of the afferent arteriole it seems likely that the juxtaglomerular cells will possess DA_1 receptors, the subtype known to be characteristic of the renal vasculature. The effects of stimulation of DA_1 receptors are believed to be mediated via adenylase cyclase and the formation of cAMP as second messenger. In a study in dogs, dibutyryl cyclic AMP was a potent stimulus to renin release (Vikse et al., 1985). Further evidence for a stimulatory affect of DA_1 receptor activation on renin release comes from work with the selective DA_1 agonist, fenoldopam. Administration in man is associated with an early and marked rise in plasma renin activity (Harvey et al., 1986). It has been suggested that the rise is a secondary response to reflex sympathetic drive, consequent upon a reduction in blood pressure (Brennan et al., 1983). However, Montier and colleagues (1987) have demonstrated in the dog that the renin response to fenoldopam was unchanged by propranolol but completely blocked by the

specific DA₁ antagonist SCH 23390.

Most studies indicate that administration of L-dopa results in suppression of renin (reviewed by Sowers, 1984). L-dopa is able to cross the blood-brain barrier, and Blair et al. (1977) have postulated a central role for dopamine in inhibition of renin, through reduction in sympathetic outflow. They administered L-dopa to anaesthetised dogs and produced an increase in renin activity. This was reversed by addition of a peripheral decarboxylase inhibitor, in the presence of which L-dopa suppressed renin, and the response was abolished by renal denervation. However, not all evidence is in agreement with this, and in rats, L-dopa increases renal noradrenaline content (Sato et al, 1987). Gludopa penetrates the brain poorly and in the present studies, the effect of carbidopa, a peripheral dopa decarboxylase inhibitor, points to inhibition of renin being a peripheral, renal event. This is supported by the study of Worth et al. (1986) who demonstrated in man that the peripheral DA₂ receptor antagonist, domperidone, blocked the renin inhibition produced by gludopa. That the DA₂ receptor mediates the inhibition of renin is supported by the study of Cavero et al. (1987), in which the DA₂ agonist quinpirole reduced PRA after infusion into intact rats.

In discussing the effect of gludopa and L-dopa on renin release, it is relevant to consider the physiological manner by which dopamine is presented to the juxtaglomerular cells. Infusion of dopamine and administration of a drug such as fenoldopam will result in very large concentrations of agonist directly to the juxtaglomerular cells. Ball et al. (1981) investigated renin release in the conscious beagle dog. Infusion of dopamine at 1.6 nmol/kg/min more than doubled plasma dopamine but produced no change in plasma renin activity. A ten-fold increase produced only a small increase in renin, and an infusion rate of 160 nmol/kg/min was required to double plasma renin levels. It is therefore

evident that circulating levels of dopamine within the physiological range, are unlikely to modulate renin release under normal conditions. Gludopa infusion results in large increases in urinary dopamine, with modest amounts entering the circulation and mimics the physiological mode of synthesis. Thus endogenous dopamine, produced in the proximal tubular cells and released into the tubular lumen, gains access to the juxtaglomerular cells by an unknown mechanism which may involve uptake by the macula densa into the microenvironment around the juxtaglomerular cells. The DA_2 receptors on presynaptic neurones are perhaps 1000 times more sensitive to dopamine than are DA_1 receptors (Kebabian and Calne, 1979). It may be therefore that relatively small amounts of dopamine, gaining access to juxtaglomerular cells after gludopa infusion, preferentially stimulate DA_2 receptors on the noradrenergic nerve endings in contact with the renin-producing cells. The result will be to inhibit noradrenaline release and hence remove a tonic stimulus to renin synthesis. The renin response following gludopa is therefore a balance between the stimulatory effect of DA_1 receptors and inhibitory effect of presynaptic DA_2 receptors. It seems clear that at doses between 12.5 and 100 $\mu\text{g/kg/min}$, inhibitory influences predominate.

One can speculate upon the dopamine-lithium interaction in the kidney with regard to renin-release. Lithium abolished the natriuresis produced by gludopa, without affecting glomerular filtration rate, a response possibly attributable to DA_1 receptor blockade at the proximal tubule. However, the reduction in plasma renin activity following gludopa was comparable in the presence or absence of lithium, suggesting that lithium is not active upon presynaptic DA_2 receptors.

The capacity of a renal dopamine prodrug to suppress renin confers great therapeutic potential. Most natriuretic and vasodilator substances, have

the tendency to increase renin by a reflex sympathetic mechanism, the effect of which is to offset drug action. An agent that is able to inhibit renin, or at least prevent reflex increases, may have a prolonged natriuretic and vasodilatory action and avoid the propensity for tachyphylaxis (false tolerance).

Interactions with other hormones

Dopamine and vasodilatory prostaglandins exert similar vasodepressor effects in the circulation, and more particularly produce renal vasodilation and natriuresis after pharmacological administration. The prostaglandins, PGE_2 and PGI_2 may mediate, at least in part, the renal action of kinins, and many influences in the kidney are known to modulate prostaglandin synthesis and release. It has therefore been questioned whether dopamine stimulates the local formation of various prostanoids in the kidney, and through this mechanism, exerts its known effects on renal function.

Previous work is scanty on the question as to whether prostaglandins are stimulated by dopamine. A recent study in man (Nadler et al., 1986) reported increased urinary prostaglandin release in response to dopamine infusion ($1 \mu\text{g/kg/min}$) but in the anaesthetised dog, intrarenal arterial dopamine did not influence PGE_2 excretion (Vikse et al., 1985). Güllner and colleagues (1982) have conversely studied the effect of alterations in prostaglandin synthesis on dopamine excretion. Chronic inhibition of prostaglandin synthesis with indomethacin (2 mg/kg/day) for seven days, did not alter urine dopamine excretion in healthy women, and dopamine output was normal in a group of patients with Bartters syndrome in which overproduction of the prostaglandins, PGE_2 and PGI_2 is a characteristic feature (Güllner, 1982).

Yehati et al. (1986) have reported that infusion of indomethacin (2 mg/kg) attenuated the renal effects produced by dopamine infused at 6

$\mu\text{g/kg/min}$ into five subjects. In a study in rats, Chevillard et al. (1978) provided evidence for a contribution of prostaglandins to the vasopressor effects of dopamine, though renal function was not assessed. Three other studies suggest that the prostaglandins and dopamine do not importantly interact within the kidney. Dressler et al. (1975) infused low dose dopamine into the renal artery of anaesthetised dogs and showed that whereas indomethacin increased the renal vasoconstrictor response to angiotensin II, it did not affect the vasodilatory response to dopamine. Similarly, Robertson et al. (1980) studied the Wistar albino rat pretreated with phenoxybenzamine and propranolol. Neither the reduction in mean arterial blood pressure nor renal vascular resistance produced by dopamine was influenced by indomethacin. Pendleton and Woodward (1976) had earlier shown in the anaesthetised dog that renal vasodilation produced by dopamine ($3 \mu\text{g/kg/min}$) was selectively inhibited by the dopamine antagonist, bulbocapnine, and that produced by PGA_1 , by indomethacin, but there were no crossover effects between the two antagonists.

The present studies provide no evidence for hormonal interactions in the kidney after pharmacological stimulation with gludopa. The dose of indomethacin chosen has previously been shown to inhibit prostaglandin excretion by at least 80% (Mackay et al., 1984) and the expected decline in plasma renin activity pointed to effective prostaglandin suppression during the course of the study. The peak responses in sodium excretion were comparable after gludopa in the presence or absence of indomethacin, and total sodium outputs over the study period were not significantly different. As discussed, prostaglandins in pharmacological doses, produce renal vasodilation and may inhibit tubular reabsorption of sodium at the distal tubule, and the system assumes greater importance during conditions of sodium depletion or reduced renal perfusion pressure. It is conceivable that under certain experimental or clinical condit-

ions, indomethacin might modify the renal effects of dopamine, by inhibiting distinct, though synergistic effects evoked by the prostaglandins at separate sites in the nephron. The study of Yehati et al. (1986) referred to, was notable for the age range of the subjects, quoted between 40 and 60 years. Renal function declines steadily after the age of 40, and such a process may be associated with greater prostaglandin-dependence of renal blood flow and glomerular filtration rate in older age groups.

Urinary prostaglandins were not measured in the present studies. Seminal vesicular fluid contains large amounts of prostaglandins and contamination of urine samples in males renders interpretation of urinary prostaglandin levels hazardous. Even after abstention from sexual intercourse for three days, enormous variations in baseline values can be recorded (McAuslane, personal communication). It is unknown at present, whether dopamine synthesis blockade, with carbidopa for instance, affects endogenous prostaglandin excretion which might provide evidence for an interaction under physiological conditions. From the present work and most reported studies, it is most likely that dopamine exerts effects on renal function by an action on specific receptors, and that the prostaglandin system is not required as a mandatory intermediary. Rather they form separate, parallel systems each of which, under certain conditions, may influence or modify the respective action of the other.

The effects of gludopa on urine kallikrein excretion were inconsistent. A small increase was apparent within 30 minutes of infusion, but levels quickly returned to baseline at a time when sodium excretion was approaching maximal values. A number of stimuli that produce diuresis are associated with increased kallikrein excretion, and in the present studies, the kallikrein response may represent a "washout" phenomenon. Mills and colleagues (1978) studied the kallikrein response to a number of vasoactive agents and postulated a natriur-

etic hormonal cascade in the kidney in which dopamine leads kallikrein production. From the present results, the kinin-kallikrein system did not participate in the natriuretic actions of gludopa, and any relationship between the two systems does not appear to be important.

With the developing interest in ANP, various groups have suggested that the renal effects of the peptide are mediated through dopamine receptors. This will be described in some detail in a further section. Gludopa did not significantly affect plasma ANP levels measured after 90 minutes of infusion. This agrees with the study reported by Shenker et al. (1987), during dopamine induced natriuresis in man. The physiological stimulus to ANP release is believed to be the degree of atrial stretch, itself dependant on central venous pressure. The constancy of plasma ANP during the infusion of gludopa therefore probably reflects the stability of the systemic parameters, blood pressure and pulse, and presumably central venous pressure.

Lithium

A simple method for the determination of segmental tubular sodium and water handling in the nephron, under in vivo conditions, has been widely sought. Maximal free water clearance, enhanced fractional urine excretion under maximal diuresis, and endogenous markers have been utilised, but the validity of results has been questioned (reviewed by Schuster and Seldin, 1985). Studies in water-loaded humans have shown a high degree of correlation between urine flow during water diuresis, and the clearance of lithium (Thomsen and Olesen, 1984). A comparable situation exists in ADH-deficient Brattleboro rats (Thomsen, 1977). In rats, micropuncture has confirmed that lithium is reabsorbed in the proximal tubule to the same extent as water and sodium, but neither reabsorbed nor secreted in the distal tubule except under

conditions of salt depletion. Thus analysis of lithium clearance in urine after single dose administration provides an estimate of fluid delivery from the proximal tubule, and comparison of the fractional clearances of lithium and sodium allows analysis of proximal and distal tubular handling of sodium (reviewed by Thomsen, 1984). The method has been used to assess the site of action in the nephron of various natriuretic agents including calcium antagonists (Krusell et al., 1986), ANP (Brown, 1986) and diuretics (Thomsen and Leyssac, 1986). However, many studies have demonstrated that lithium itself alters renal electrolyte handling. Effects produced by the administration of lithium have been variable and confusing, both sodium depletion and retention having been described. These variable findings may relate to prevailing salt status, the animal model, acute versus chronic lithium administration, and whether toxicity was induced (Myers et al., 1980; Singer, 1981).

Under the conditions of this study, lithium was natriuretic at around 12 hours after ingestion. Previous studies in humans (Murphy et al., 1969; Baer et al., 1971) have also demonstrated acute natriuresis during the first 24 hours of lithium administration followed by sodium retention over the succeeding days. Martinez-Maldonado et al. (1975) demonstrated in rats that lithium increased the renal excretion of urate and phosphate, both principally reabsorbed in the proximal tubule, and micropuncture suggests that inhibition of sodium reabsorption affects all segments of the nephron (Hecht et al., 1978). The mechanism of the effect is unknown. Thompson et al. (1984) infused lithium into rats and ascribed part of the natriuresis to expansion of the extracellular fluid volume. This cannot be a factor in the present study as the total dose administered was only 20 mmol. Lithium can partially substitute for sodium and potassium in the intracellular space and may affect transport mechanisms. For instance, in the brain, lithium has been demonstrated to inhibit Na^+/K^+ -ATPase

(Guerri et al., 1981) and the Na^+/Li^+ countertransport mechanism is inhibited by lithium in red blood cells (Ehrlich et al., 1983). In addition, there is evidence that lithium can induce a relative resistance to the action of aldosterone on the distal nephron (Singer, 1981). In a recent report, an increased requirement for mineralocorticoid replacement was demonstrated in a patient with Addison's disease requiring concurrent lithium therapy (Stewart et al., 1987).

The increased plasma renin activity produced by lithium has been reported previously in animals (Nally et al., 1980) and man (Shopsin et al., 1973). The blood pressure was unchanged by the administration of lithium arguing against an appreciable stimulus to the renal baroreceptor or change in sympathetic tone. In the study reported by Murphy et al. (1969), the salt deficit over the first 24 hours of lithium averaged 32 mmols, an amount unlikely to be important in the present salt replete subjects. In addition, the increased sodium load at the macula densa would be expected, if anything, to inhibit renin release. In fact, Nally et al. (1980) have argued that the acute increase in plasma renin activity produced by lithium in dogs represents a direct effect at the juxtaglomerular cells.

Paradoxically, after producing an increase in control sodium output, lithium, at low serum concentration, markedly attenuated the natriuresis produced by gludopa, and in fact sodium excretion did not significantly increase. A number of possibilities exist for this effect. It seems unlikely, as discussed above, that the small sodium deficit produced by lithium would alter the renal responsiveness to gludopa. However, the elevated PRA levels before and during the infusion were likely to have been accompanied by raised angiotensin II levels. Angiotensin II is antinatriuretic and has a direct action at the proximal tubule (Schuster et al., 1984a). Interactions between angiotensin II and dopamine have not so far been reported and further studies will be

required on this possibility, perhaps using angiotensin converting enzyme inhibitors. The effect of lithium on the phosphaturic and uricosuric actions of gludopa are of interest. The increase in urate excretion was blocked by lithium in a manner analogous to that of sodium. Lithium did not significantly affect the marked phosphaturic response, further evidence that the renal handling of phosphate, and modulation by dopamine, is not directly linked to that of the sodium ion.

Lithium is known to have marked effects on adenylyl cyclase activity. The cAMP responses to vasopressin, glucagon and parathyroid hormone are blocked by lithium (Waller et al., 1983 and 1984) and it has also been demonstrated to block dopamine induced cAMP release in the brain (Geisler and Klysner, 1985). Lithium did not alter GFR, and it seems reasonable to postulate that lithium attenuates the natriuretic capacity of gludopa by interfering with cAMP release at the proximal tubule. The results presented for urine cAMP release do not provide direct evidence for this, and the difficulties in assessing urinary cAMP values has been discussed. It is of relevance that lithium has also been demonstrated to interfere with the action of preformed cAMP on the renal tubule (Forrest et al., 1974). Further studies are required on the effect of lithium on dopamine induced cAMP release in the kidney, ideally using isolated tubule preparations.

Much interest is being generated in the role of the phosphoinositide system as an intracellular second messenger. The recent demonstration that both dopamine and lithium have actions upon different parts of this system, dopamine stimulating phospholipase C (Felder et al., 1987) and lithium inhibiting inositol di- and mono-phosphatase (Drummond, 1987), opens up a new area of potential interaction that awaits clarification.

Gludopa did not significantly increase lithium clearance although there

was a tendency towards a rise. Two points however deserve mention. Firstly, the natriuresis was small and insignificant in the presence of lithium so that the increase in lithium clearance was within the background error of the observations. Secondly, to use lithium as a marker presumes it to be pharmacologically inert which was patently not the case. The clearance results may therefore be uninterpretable and the lithium clearance method not suitable for the investigation of dopaminergic mechanisms in the kidney. It will also be of great interest to attempt to establish whether the acute effects of lithium seen in this study can also be observed in patients on long term lithium therapy or whether some form of adaptation takes place.

CHAPTER FOUR

STUDIES ON THE URINE DOPAMINE RESPONSE TO FRUSEMIDE IN NORMAL MAN

INTRODUCTION

The loop diuretic frusemide is a powerful natriuretic agent with its principle action at the ascending limb of the loop of Henlé. An increase in renal blood flow and plasma renin activity are intimately associated with its action. Kuchel et al. (1979) reported that intravenous frusemide acutely stimulated urine dopamine excretion in man, although the mechanism was not investigated, nor, in detail, the time course of the response. The group also demonstrated that the dopamine and natriuretic response to frusemide was reduced in essential hypertension. This is a finding of some interest. As discussed, a relative dopamine deficiency in response to salt loading may identify those with a predisposition to "salt-sensitive" hypertension: the response to frusemide may provide a simple method to discriminate between those with impaired dopamine regulation compared to normal subjects. However in this department, a short preliminary study (Rutter, unpublished) suggested, in normal subjects, that dopamine excretion actually declined after the oral administration of frusemide.

The first aim of the present group of studies was to further characterise the dopamine response to oral frusemide, in relation to the time course of sodium output and urine flow. Thereafter, the response was compared in the same subjects given the same dose of frusemide intravenously.

The dose of frusemide chosen has been demonstrated to stimulate prostaglandin release from the kidney, and a number of the effects of frusemide are believed to be mediated through the prostaglandins, although there remains some controversy (Carmichael and Shankel, 1985). The cyclooxygenase inhibitor, indomethacin, was administered in the second part to assess whether the dopamine response to frusemide is similarly related to, and dependant on,

prostaglandin release.

Dopamine, in terms of its ability to produce renal vasodilation and natriuresis, mimics certain of the effects of frusemide. In the final part, the renal responses to frusemide were compared in the presence or absence of the dopa decarboxylase inhibitor, carbidopa, to determine whether dopamine plays any part in its natriuretic and hormonal effects.

2(a) THE EFFECT OF ORAL FRUSEMIDE ON URINE DOPAMINE EXCRETION

Protocol

Nine subjects were studied on a single occasion. Frusemide (30 mg) was given by mouth after a 2 hour run-in period. The study continued for a further 5 hours. Urine and blood samples, and blood pressure and pulse measurements were taken at 30 minute intervals except for two 15 minute intervals in the first 30 minutes after frusemide was given. Two subjects were unable to consistently pass urine at required times and their results are not included. Control results were those for the final 30 minutes of the run-in period.

Results

Figure 2a-1 shows the natriuretic and urine flow response to frusemide in seven subjects. An increase in both parameters was evident at the first collection point at 15 minutes, though maximal responses were not, on average, attained until 1-1½ hours after administration. There was however marked variation between individuals in terms of the magnitude of, and time to, peak responses, which is evident from the size of the error bars. Diuresis closely paralleled the natriuresis, and in all subjects the responses were completed within 5 hours of administration of frusemide. Plasma renin activity increased gradually during the course of the study, and levels were significantly elevated above control from 3½ hours (Figure 2a-2). Haematocrit values however showed a steep and early rise, reaching a maximum at 2 hours, and thereafter tending to decline. Mean blood pressure and pulse rates were not significantly altered from control throughout the study (Table 2a-1). Urine dopamine excretion increased transiently though significantly at 15 minutes, the rise being of the order of 40% and occurring in all subjects. However, levels had returned to

baseline by 30 minutes, a point at which urine flow and sodium output were still increasing. Dopamine excretion tended to fall thereafter and at $3\frac{1}{2}$ hours, was significantly below control (Figure 2a-3).

	Time (hours)							
	C	$\frac{1}{4}$	$\frac{1}{2}$	1	2	3	4	5
Mean arterial pressure (mm Hg)	81.7 + 2.3	82.1 + 1.5	83.6 + 1.9	86.0 + 2.1	85.4 + 3.0	85.9 + 1.8	82.7 + 6.2	81.1 + 1.9
Mean pulse rate (beats/min)	58 + 2	57 + 2	59 + 3	59 + 3	59 + 3	59 + 1	60 + 3	60 + 2
Haematocrit (%)	42.4 + 1.1	42.4 + 1.1	43.3 + 0.9	44.3* + 1.0	44.6** + 1.0	44.4** + 0.9	43.9 + 1.1	44.5** + 0.8

Table 2A-1 Mean blood pressure, pulse and haematocrit values in response to frusemide (30 mg) by mouth in seven subjects. Values are means \pm SEM; * $p < 0.05$, ** $p < 0.01$ in comparison with control.

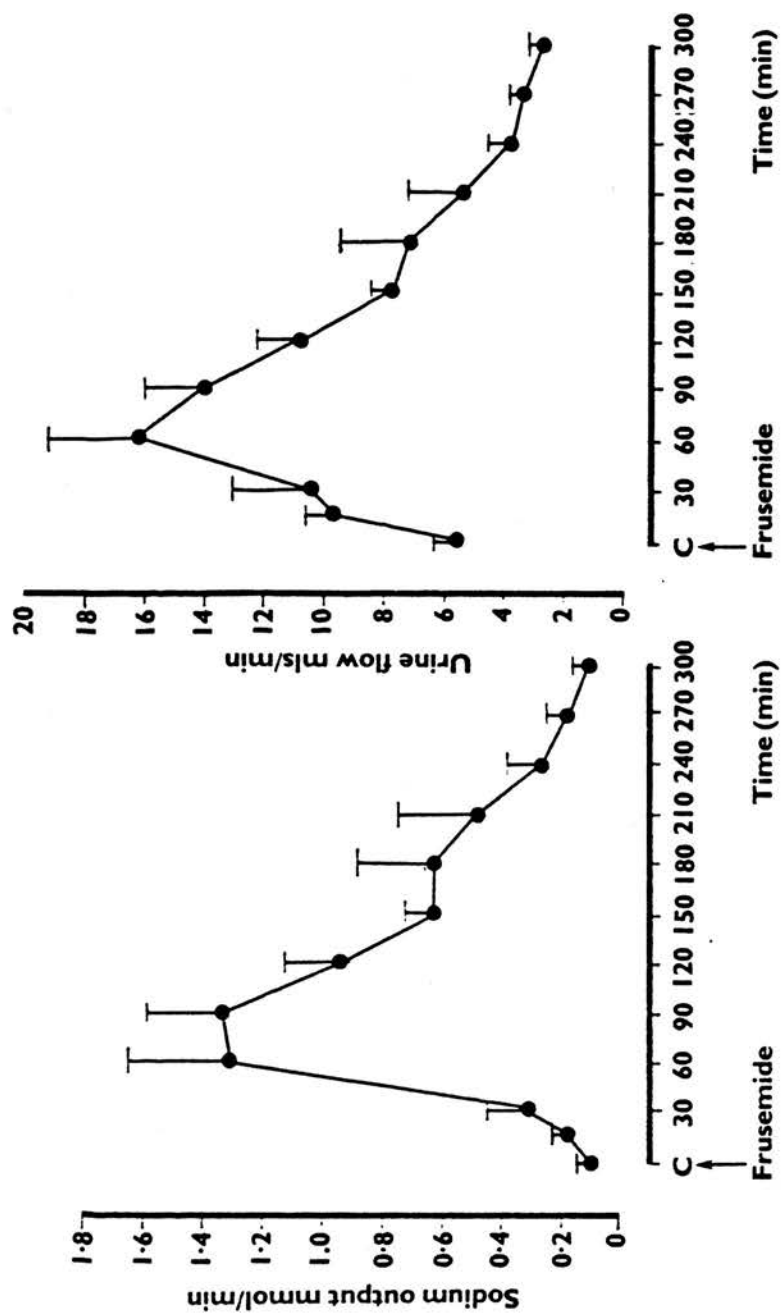


Figure 2a-1. Urine sodium and volume output in response to oral frusemide (30mg) in seven subjects. Values are means \pm SEM.

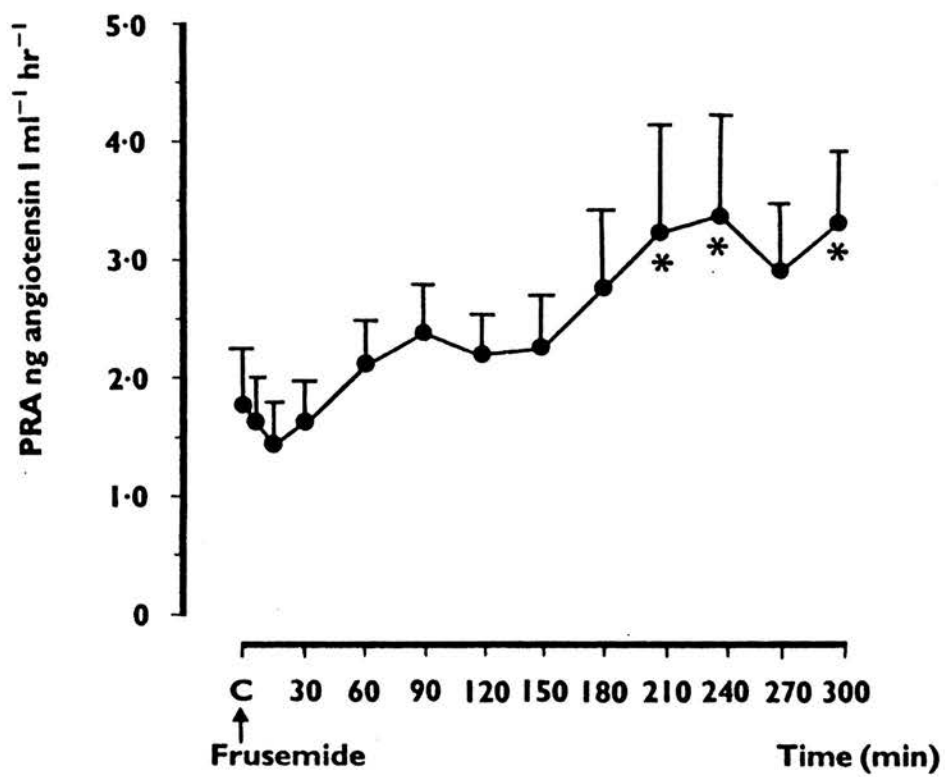


Figure 2a-2. Plasma renin activity in response to oral frusemide (30mg) in seven subjects. Values are means \pm SEM, * $p < 0.05$ in comparison with control.

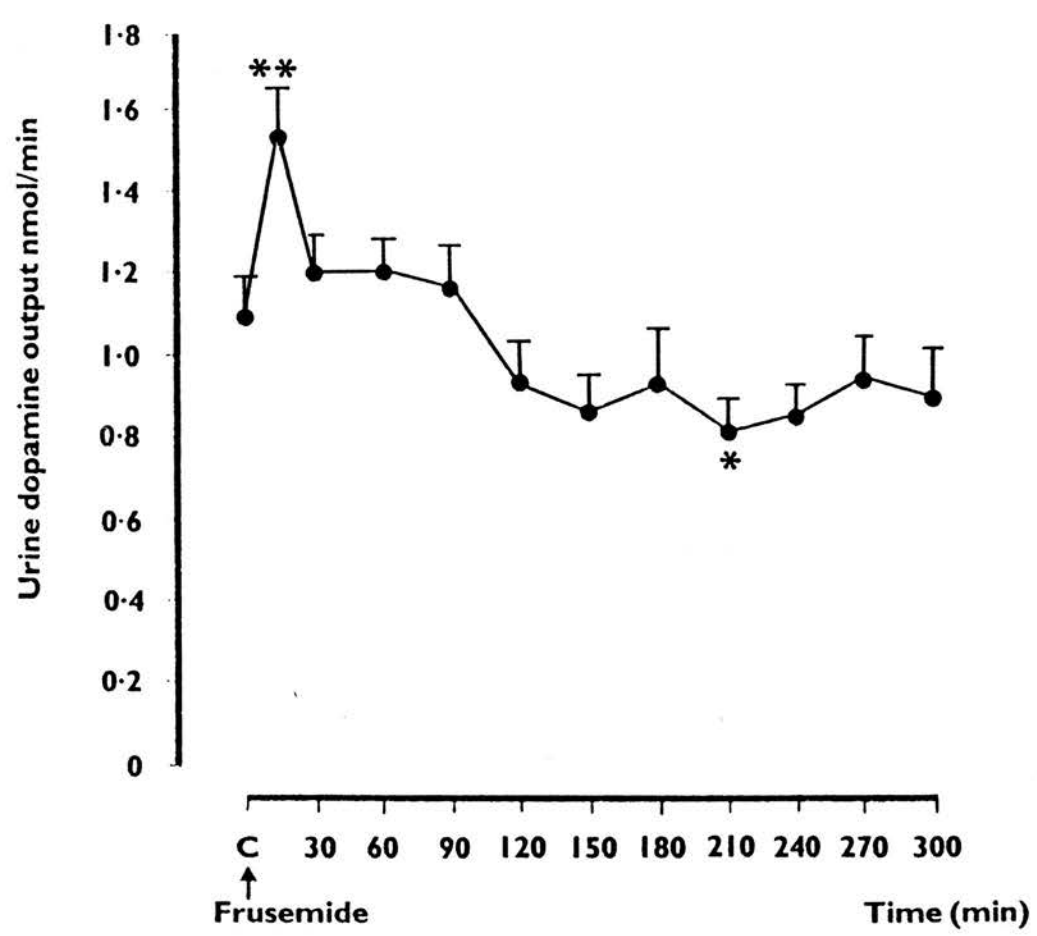


Figure 2a-3. Urine dopamine output in response to oral frusemide (30mg) in seven subjects. Values are means \pm SEM, ** $p < 0.01$ * $p < 0.05$ in comparison with control.

2(b) THE EFFECT OF INDOMETHACIN ON THE URINE DOPAMINE RESPONSE TO INTRAVENOUS FRUSEMIDE

Protocol

Seven volunteers were each studied on two occasions. A 2 hour run-in period (-2 to 0 hr) was followed by an intravenous bolus of frusemide (30 mg) over one minute. The study then continued for a further 3 hours. At $-1\frac{1}{2}$ hrs either placebo or indomethacin (100 mg) was given by mouth. Urine and blood sampling, and blood pressure and pulse measurements were taken at -1, $-\frac{1}{2}$, 0, $\frac{1}{4}$, $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, $2\frac{1}{2}$, and 3 hours. For statistical analysis, the results for the last $\frac{1}{2}$ hr before the administration of frusemide were taken as control (0 hr). After each study, cumulative salt loss above baseline was replaced by sodium chloride tablets. In addition, 3 volunteers undertook two further study days, taking indomethacin (100 mg) and placebo respectively. The protocol was identical to that above except that frusemide was not given.

Results

Baseline determinations

For each individual, total dopamine, sodium and volume output were calculated during the 2 hour baseline-period. Correlation analysis was not undertaken due to the small number of individuals. The mean and SEM for the ratios of dopamine:sodium and dopamine:urine flow rate were calculated (6.64 ± 0.43 nmol dopamine/mmol Na vs 0.17 ± 0.03 nmol dopamine/ml). The much lesser variability for the former suggests that dopamine output is more closely linked to sodium excretion than to urine flow rate. A comparison of values at time 0 hr, before frusemide was given, indicated that urine volume, sodium excretion and plasma renin activity, were all lower after indomethacin, but

only the results for volume reached significance ($p < 0.05$). Indomethacin did not alter urine dopamine output at this point (1.00 ± 0.16 vs 1.03 ± 0.11 nmol/min, N.S.). The results for the three subjects studied separately without frusemide are shown in Figure 2b-1. Statistical comparison was not undertaken but urine volume, sodium and plasma renin activity, were consistently reduced by indomethacin, compared with placebo. However, there was no apparent change for dopamine over the 3 hour period.

Responses to intravenous frusemide

Table 2b-1 shows the results of renal responses to intravenous frusemide. Maximal values all occurred at 15 minutes. Sodium output increased 20-fold on average with a parallel increase in urine flow rate (Figures 2b-2, 3). Natriuresis and diuresis were complete in 3 hours. Plasma renin activity showed a 4-fold rise at 15 minutes. Levels thereafter declined to plateau significantly above control until the end of the study (Figure 2b-5). Urine dopamine excretion more than doubled within the first 15 minutes, though levels thereafter started to decline and by $1\frac{1}{2}$ hours were not significantly above baseline.

Effects of indomethacin on responses to intravenous frusemide

Sodium excretion and urine flow rates in response to frusemide were reduced at all times after pretreatment with indomethacin (Figures 2b-2, 3). Comparison of total sodium outputs over the three hours after frusemide revealed a 25% reduction overall (199.2 ± 11.7 to 142.7 ± 10.2 mmol, $p < 0.01$, Figure 2b-4). Total volume output was similarly reduced (1376 ± 78 to 999 ± 92 mls, $p = 0.01$). The PRA response to frusemide was entirely abolished by indomethacin (Figure 2b-5). From Figure 2b-6 it is evident that the urine dopamine response to frusemide after indomethacin was similar to that

observed with placebo and the results are almost superimposed.

Systemic responses

Table 2b-2 shows the results for mean arterial blood pressures and pulse rates. In the presence of indomethacin, arterial pressure was higher at all points compared to placebo, and this was significant during the control period. Frusemide with placebo, resulted in a significant increase in blood pressure at 15 minutes. Thereafter there was a non-significant decline. Pulse rate tended to be lower in the presence of indomethacin, and values declined during the study. Frusemide produced a large and early increase in haematocrit, starting at 15 minutes and values remained elevated. In the presence of indomethacin the initial haematocrit tended to be higher in comparison with placebo, though the increase after frusemide was less marked.

	Sodium excretion (mmol/min)	Urine flow (ml/min)	PRA (ng of ANG I h ⁻¹ ml ⁻¹)	Dopamine excretion (nmol/min)
Before frusemide	0.15 ± 0.02	3.50 ± 0.30	1.04 ± 0.22	1.00 ± 0.16
15 min after frusemide	3.57 ± 0.14	17.00 ± 0.80	4.40 ± 0.95	2.27 ± 0.26
Significance	P < 0.001	P < 0.001	P < 0.01	P < 0.01

Table 2B-1 Sodium excretion, urine flow and hormone values before and 15 minutes after 30 mg of frusemide intravenously. Values are expressed as means ± SEM; n = 7.

	Time (hours)					
	C	$\frac{1}{4}$	$\frac{1}{2}$	1	2	3
Frusemide and placebo						
Mean arterial pressure (mm Hg)	83.6 + 3.2	89.3* + 2.8	86.9 + 2.9	86.1 + 2.2	83.1 + 0.9	82.9 + 2.0
Mean pulse rate (beats/min)	59 + 3	60 + 2	59† + 3	62† + 3	60† + 3	60 + 2
Haematocrit (%)	40.7 + 1.8	43.3** + 1.4	43.6** + 1.6	44.3** + 1.4	43.1** + 1.7	43.5** + 1.4
Frusemide and indomethacin						
Mean arterial pressure (mm Hg)	89.7† + 2.5	89.4 + 3.1	88.9 + 3.3	87.0 + 2.8	84.4 + 1.7	85.7 + 2.8
Mean pulse rate (beats/min)	57 + 2	55 + 3	53 + 2	52* + 2	53 + 2	57 + 2
Haematocrit (%)	43.0 + 1.1	43.9 + 1.1	44.7* + 1.1	44.9** + 1.1	44.6** + 1.0	44.3 + 1.4

Table 2B-2 Mean blood pressure, pulse and haematocrit values in seven subjects in response to intravenous frusemide (30 mg), after pretreatment with either placebo or indomethacin (100 mg). Values are means + SEM; * $p < 0.05$, ** $p < 0.01$ for comparison with control; † $p < 0.05$ for comparison between the effect of placebo and indomethacin.

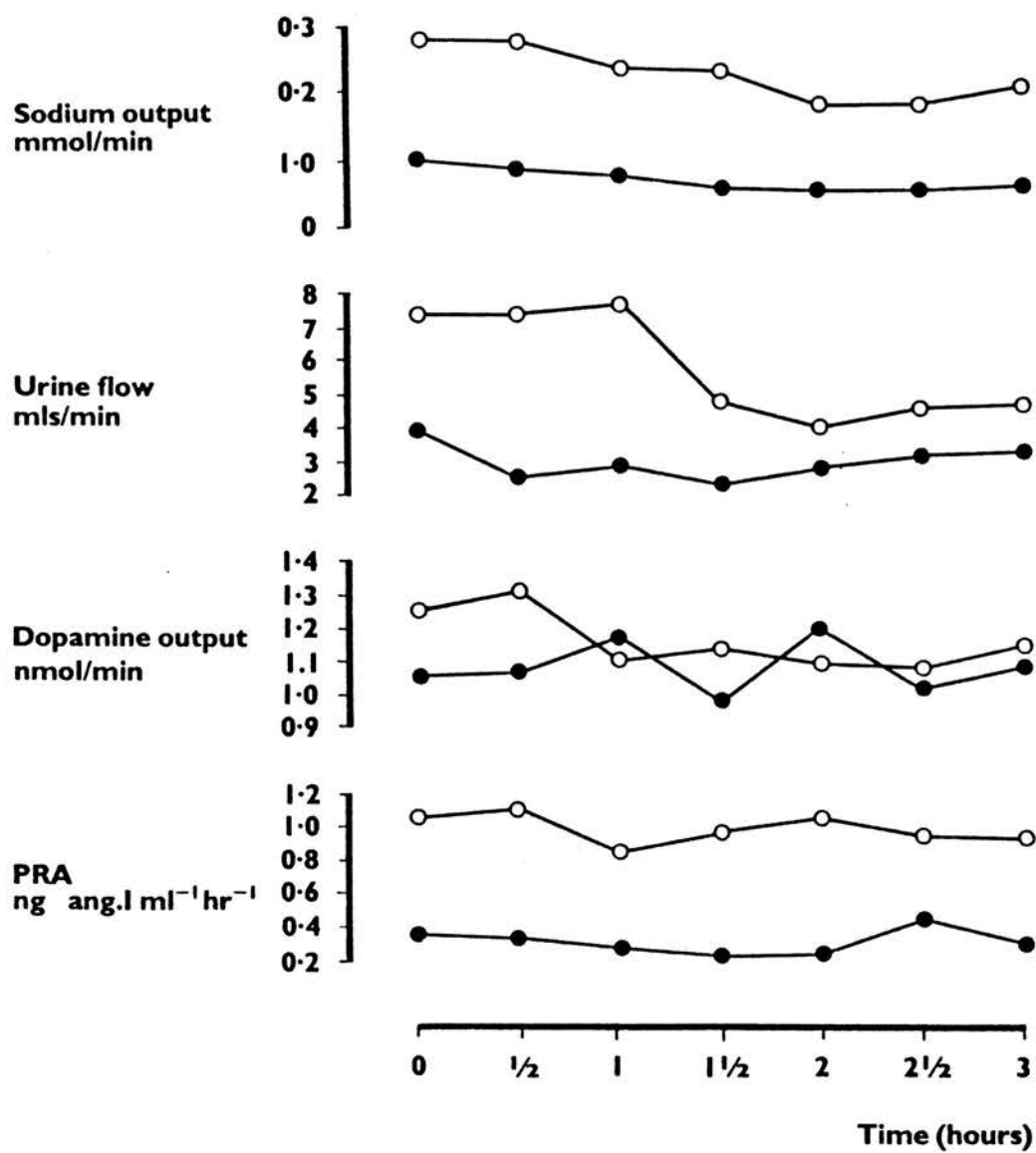


Figure 2b-1. Mean renal responses in three subjects given placebo (○) or indomethacin (100mg, ●) at time - 1½ hr.

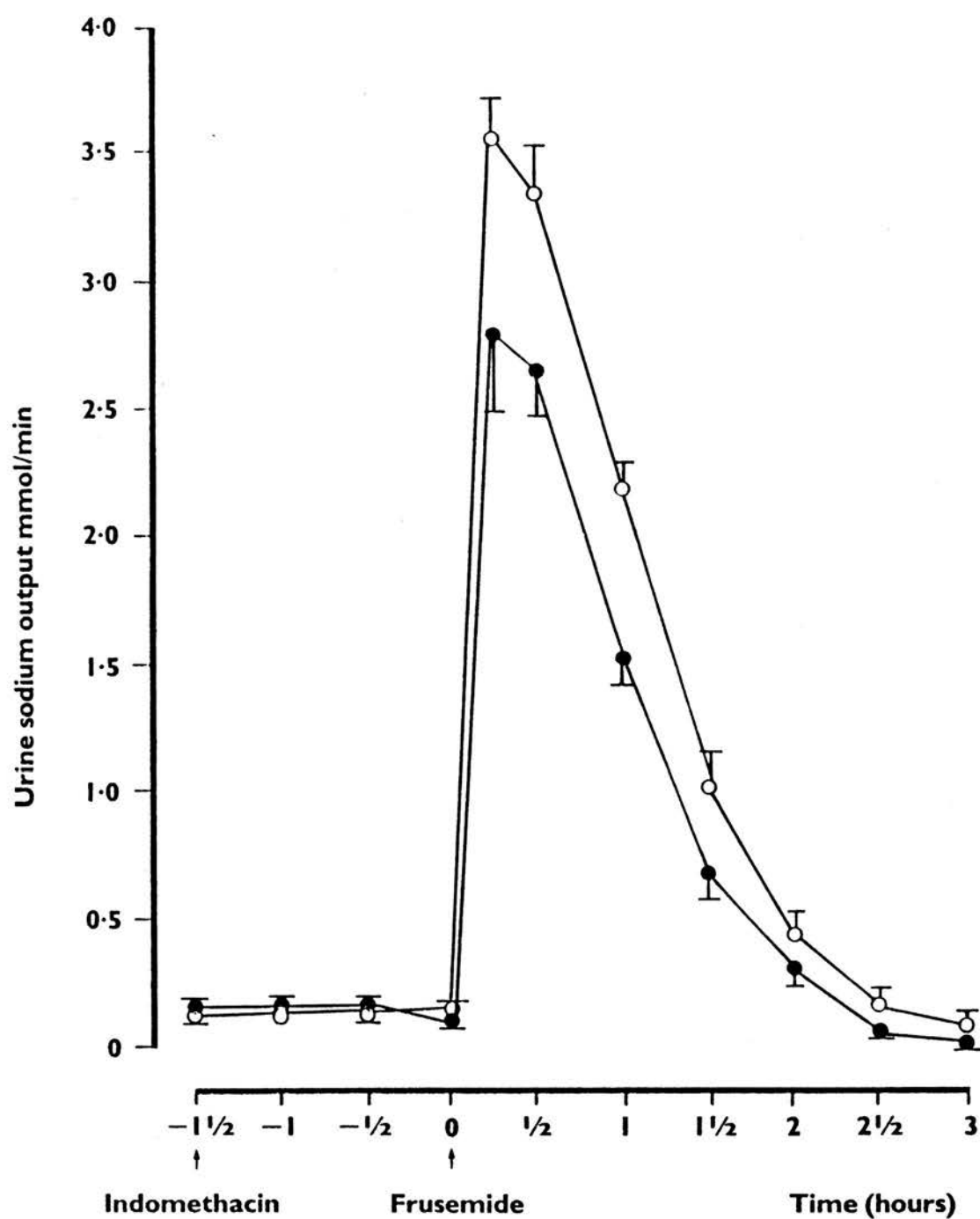


Figure 2b-2. Urine sodium output in response to intravenous frusemide (30mg) with (●) or without (○) pretreatment with indomethacin (100mg) in seven subjects. Values are means \pm SEM.

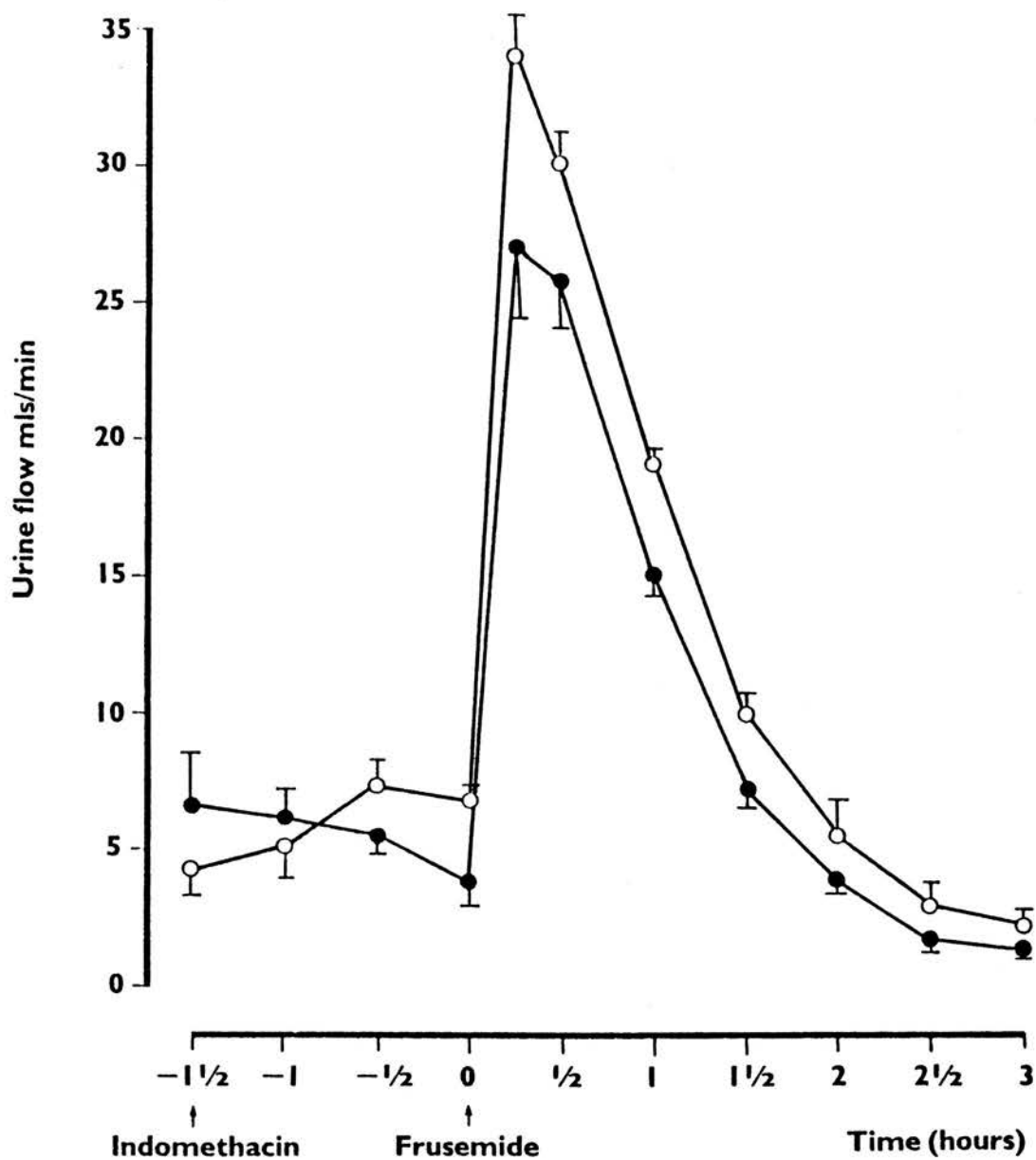


Figure 2b-3. Urine flow in response to intravenous frusemide (30mg) with (●) or without (○) pretreatment with indomethacin (100mg) in seven subjects. Values are means \pm SEM.

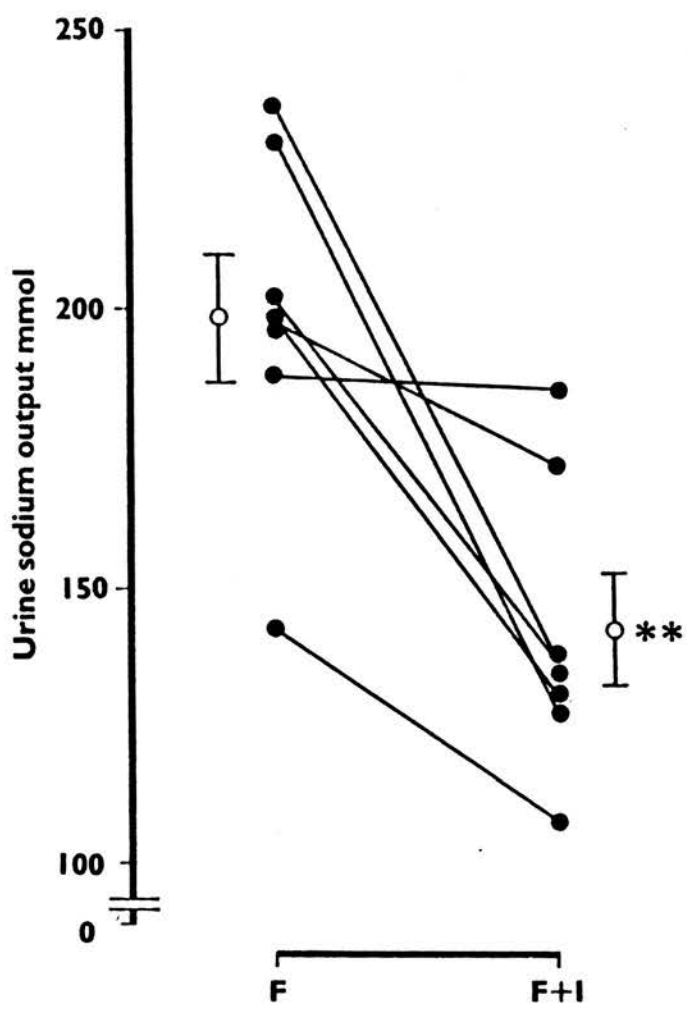


Figure 2b-4. Total natriuretic response in seven subjects, to 30mg intravenous frusemide, alone (F), and after pretreatment with 100mg indomethacin (F+I). Individual values are plotted with means \pm SEM, ** $p < 0.01$.

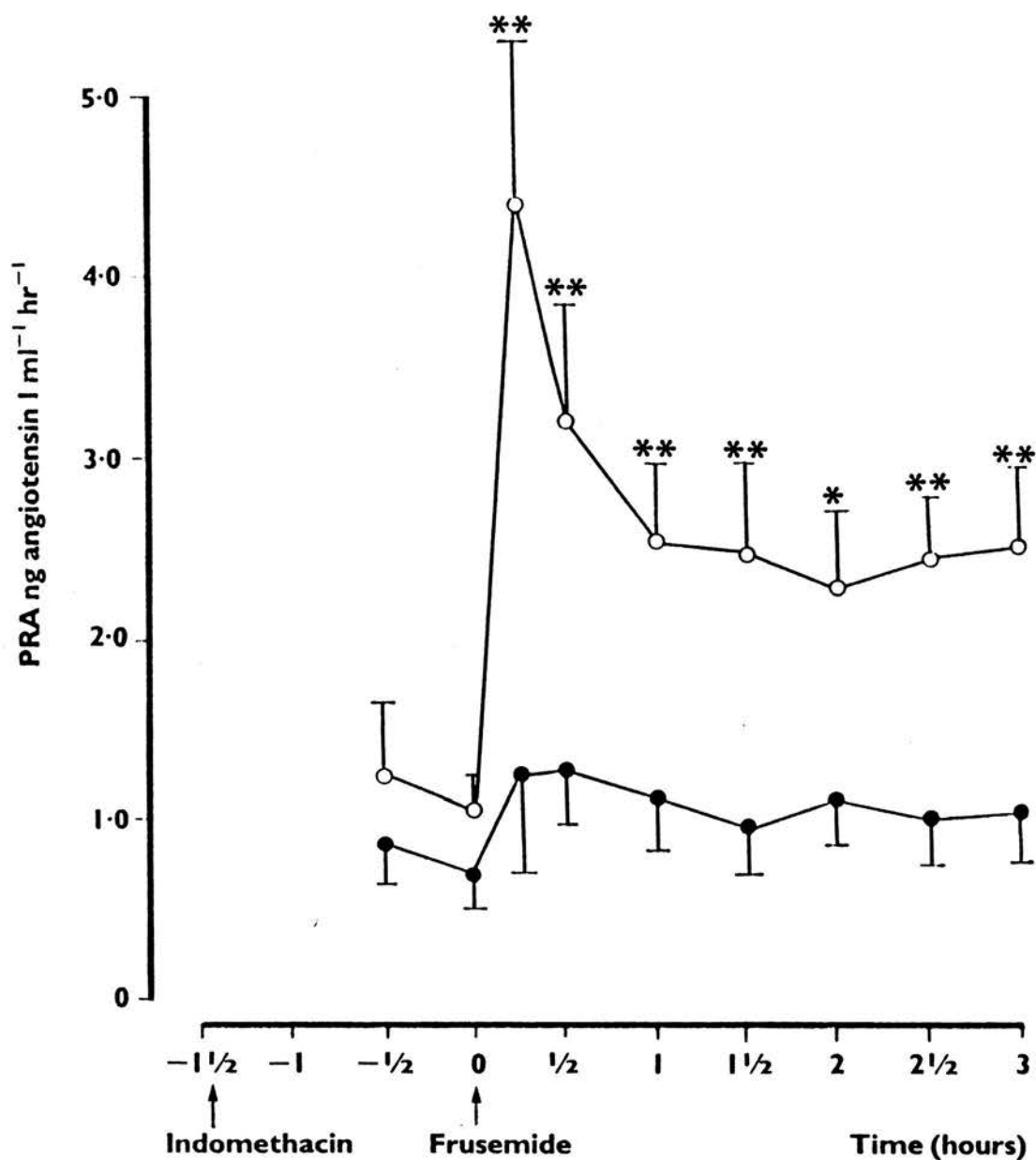


Figure 2b-5. Plasma renin activity in response to intravenous frusemide (30mg) with (●) or without (○) pretreatment with indomethacin (100mg) in seven subjects. Values are means \pm SEM, * $p < 0.05$, ** $p < 0.01$ in comparison with control (0 hr).

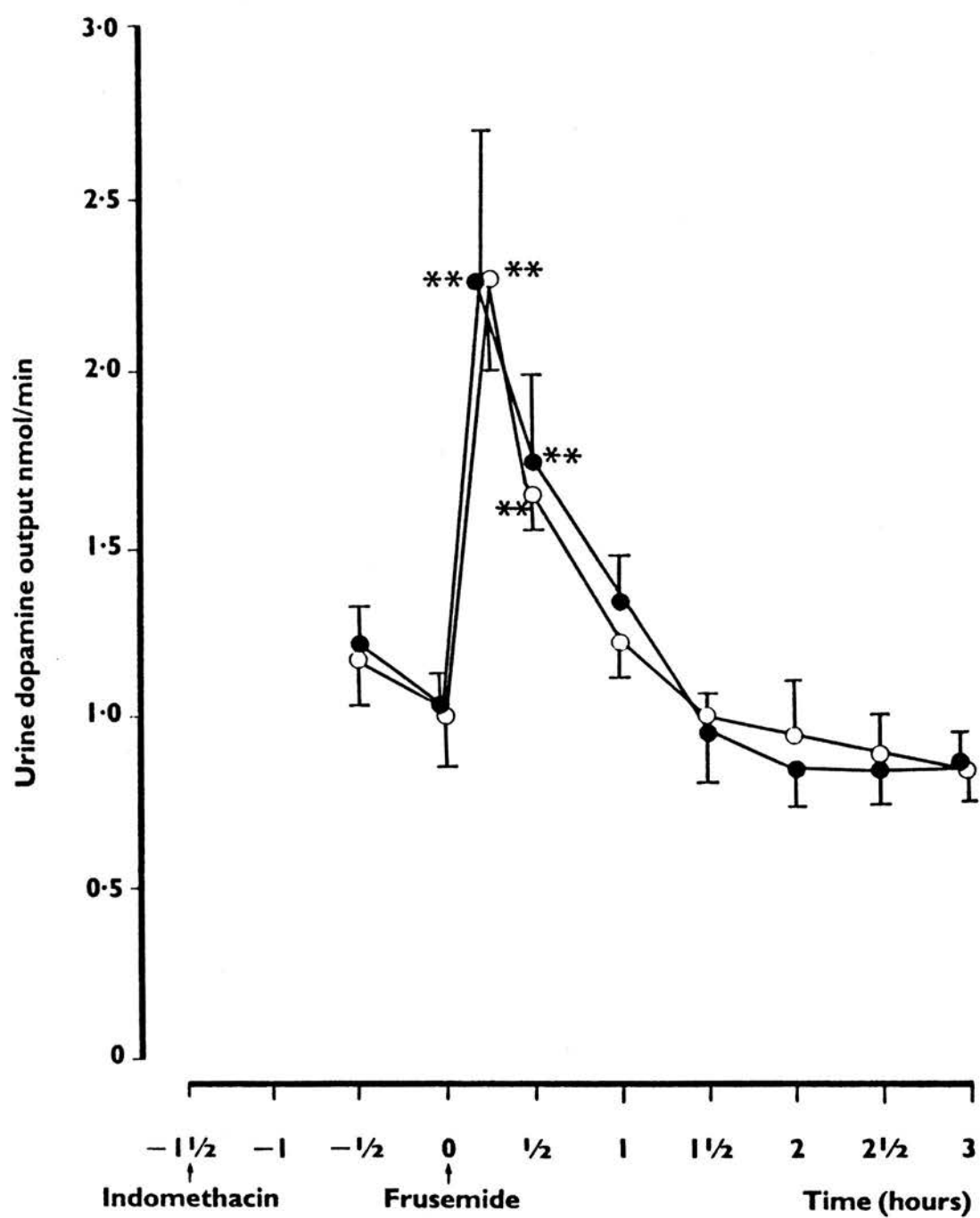


Figure 2b-6. Urine dopamine output in response to intravenous frusemide (30mg) with (●) or without (○) pretreatment with indomethacin (100mg) in seven subjects. Values are means \pm SEM, * $p < 0.05$, ** $p < 0.01$ in comparison with control (0 hr).

2(c) THE EFFECT OF CARBIDOPA ON THE RENAL RESPONSE TO INTRA- VENOUS FRUSEMIDE

Protocol

Eight volunteers were studied on two occasions. After a 2 hour equilibration period (-4 to -2 hr) carbidopa (100 mg) or placebo was given by mouth. A further two hour period was then followed by an intravenous bolus of frusemide (30 mg) (0 hr). Blood and urine samples and blood pressure and pulse measurements were taken at -2, -1, 0, $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, and 3 hours. Cumulative salt loss was replaced at the end of the study. For statistical analysis, the results for the last hour before the administration of frusemide were taken as control (0 hr).

Results

Baseline determinations

Carbidopa reduced dopamine excretion from 1.25 ± 0.12 nmol/min to the lower level of detection of the assay by two hours (Figure 2c-1). It did not produce any changes in baseline sodium excretion (Figure 2c-2), plasma renin activity (Figure 2c-4), or urine kallikrein excretion (Figure 2c-5), compared to placebo at time 0 hr.

Effect of carbidopa on responses to intravenous frusemide

Frusemide, with placebo, produced similar and comparable increases in sodium and dopamine output, and plasma renin activity to those recorded in part 2b. In addition, frusemide stimulated urine kallikrein excretion at 15 minutes (0.53 ± 0.08 to 0.94 ± 0.11 Eu/hr, $p < 0.01$), but the response was not sustained and levels had declined to baseline at 30 minutes.

In the presence of carbidopa, dopamine excretion remained below the limit of detection and no rise occurred after frusemide. There was a mean 16% reduction in the total natriuretic response to frusemide. This was largely accounted for by one subject who showed a marked decline. Three subjects produced a greater natriuretic response, and as a result, the overall change was not significant (182.4 ± 11.8 vs 156.0 ± 11.8 mmol, Figure 2c-3). The pattern and time course of the stimulation of plasma renin activity after frusemide was almost identical after pretreatment with either placebo or carbidopa (Figure 2c-4). Similarly, the rise in urine kallikrein excretion was not significantly altered by blockade of dopamine synthesis (Figure 2c-5).

Systemic responses

Frusemide produced an increase in mean blood pressure at 15 minutes which was not quite significant, but did not alter pulse rate throughout the study period.

In the presence of carbidopa, there was a significant increase in mean arterial pressure, one hour after frusemide, compared to control. There were however, no significant differences between placebo and carbidopa, on responses to frusemide (Table 2c-1).

	Time (hours)					
	C	$\frac{1}{4}$	$\frac{1}{2}$	1	2	3
Frusemide and placebo						
Mean arterial pressure (mm Hg)	88.1 + 4.0	92.9 + 3.8	91.9 + 3.5	90.4 + 4.0	89.0 + 3.7	90.3 + 4.3
Mean pulse rate (beats/min)	63 + 4	57 + 2	58 + 2	61 + 3	63 + 2	62 + 3
Frusemide and carbidopa						
Mean arterial pressure (mm Hg)	86.1 + 2.6	90.4 + 2.6	90.0 + 2.1	93.0** + 2.6	88.7 + 2.7	87.2 + 2.9
Mean pulse rate (beats/min)	62 + 3	58 + 3	59 + 2	64 + 2	64 + 3	63 + 3

Table 2C-1 Mean blood pressure and pulse in eight subjects in response to intravenous frusemide (30 mg) after pretreatment with either placebo or carbidopa (100 mg). Values are means \pm SEM; * $p < 0.05$, ** ; < 0.01 for comparison with control.

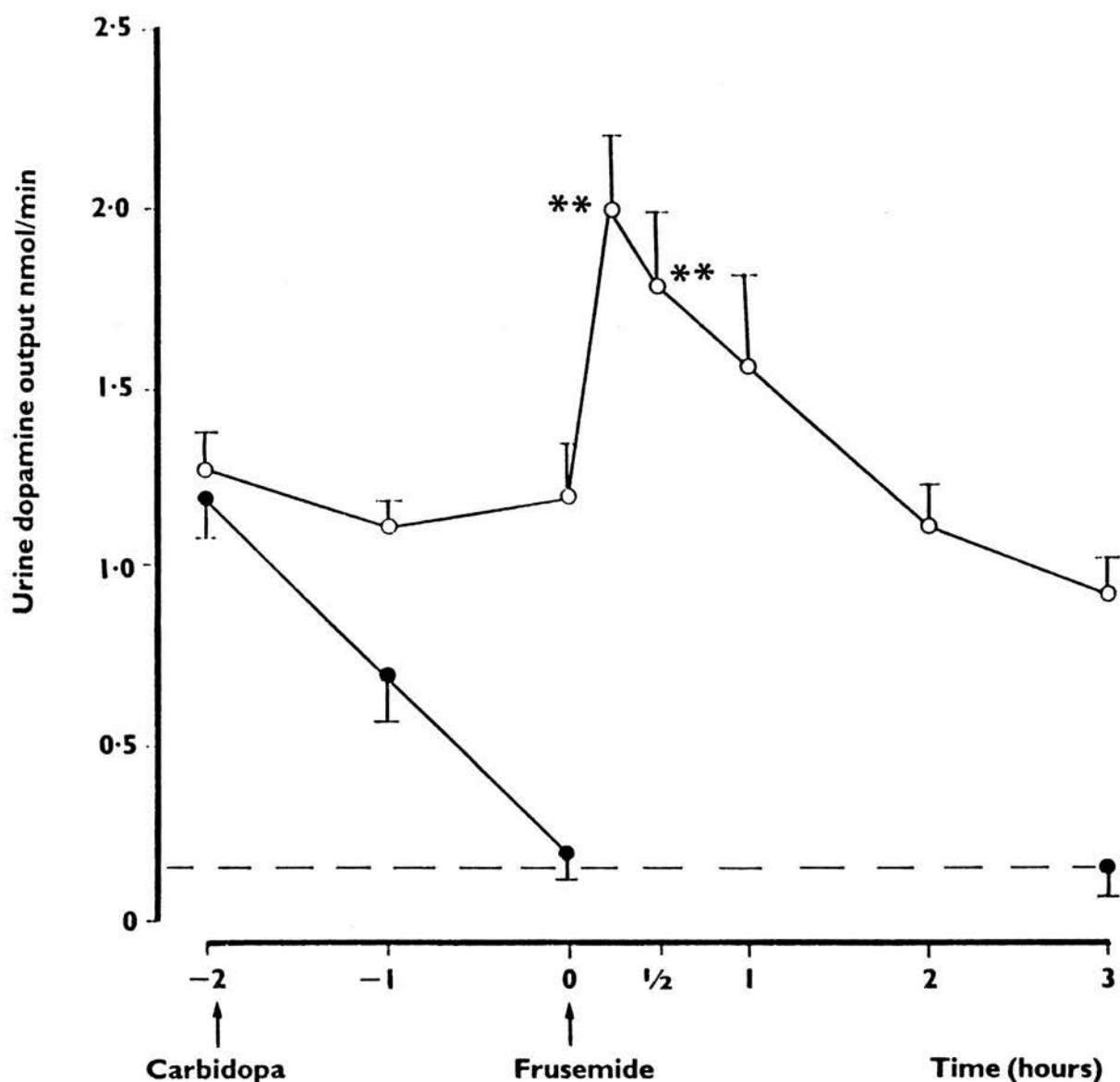


Figure 2c-1. Urine dopamine output in response to intravenous frusemide (30mg) with (●) or without (○) pretreatment with carbidopa (100mg) in eight subjects. Values are means \pm SEM, * $p < 0.05$, ** $p < 0.01$ in comparison with control (0 hr). The broken line represents the lower limit of detection of dopamine.

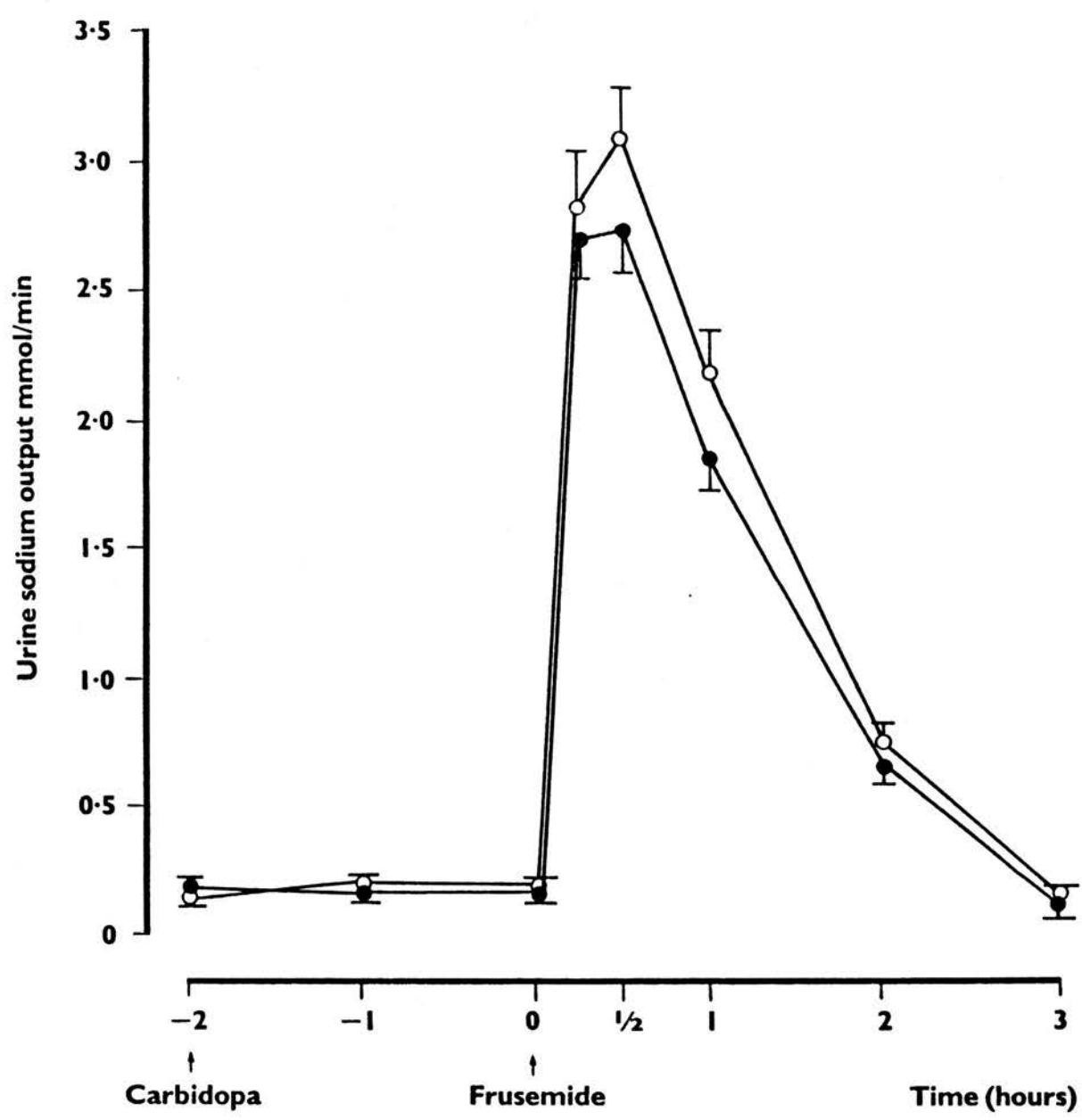


Figure 2c-2. Urine sodium output in response to intravenous frusemide (30mg) with (●) or without (○) pretreatment with carbidopa (100mg) in eight subjects. Values are means \pm SEM.

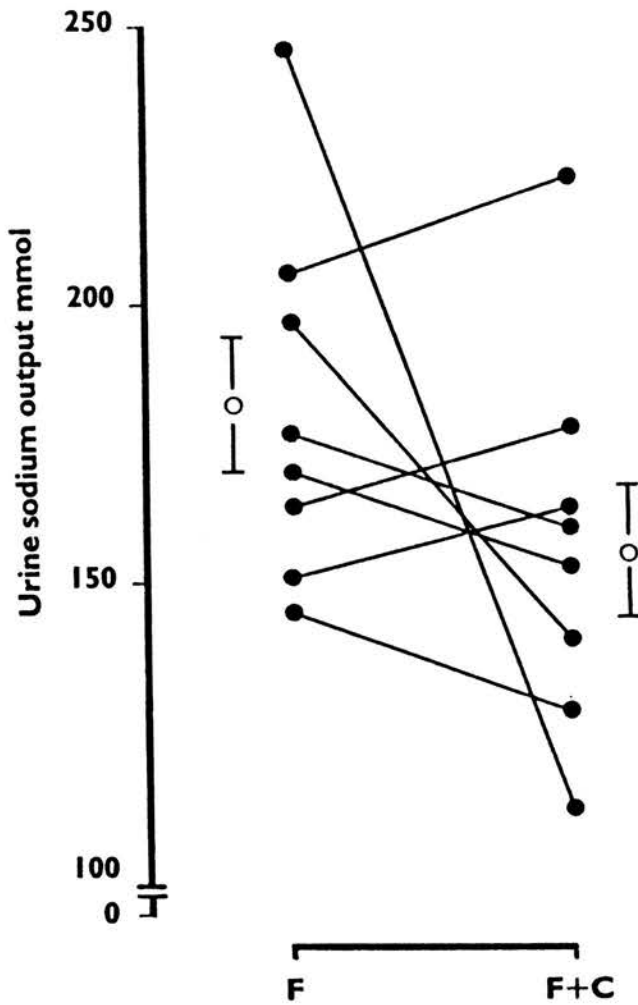


Figure 2c-3. Total natriuretic response, in eight subjects, to 30mg intravenous frusemide, alone (F) and after pretreatment with 100mg carbidopa (F+C). Individual values are plotted with means \pm SEM.

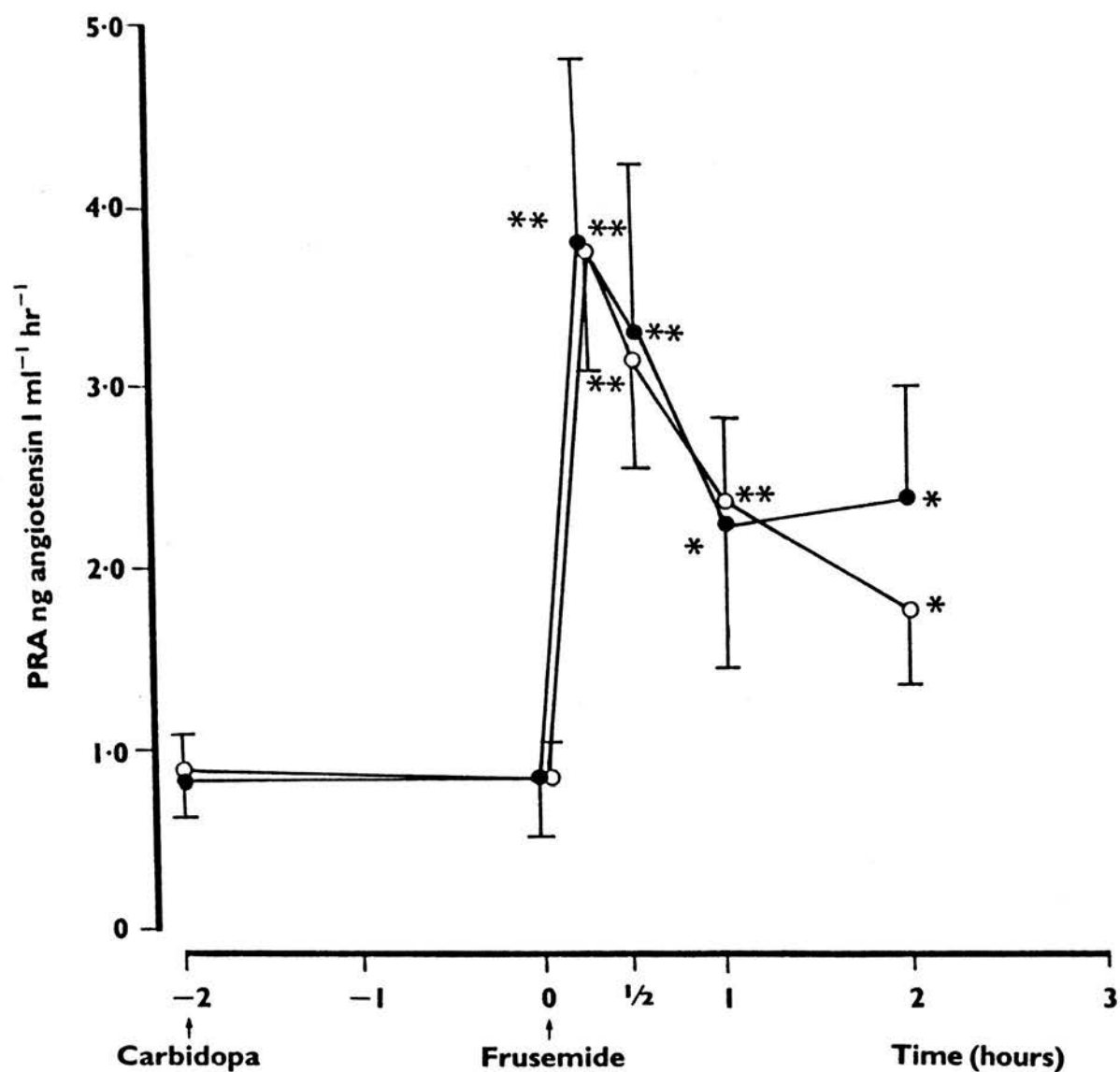


Figure 2c-4. Plasma renin activity in response to intravenous frusemide (30mg) with (●) or without (○) pretreatment with carbidopa (100mg) in eight subjects. Values are means \pm SEM, * $p < 0.05$, ** $p < 0.01$ in comparison with control (0 hr).

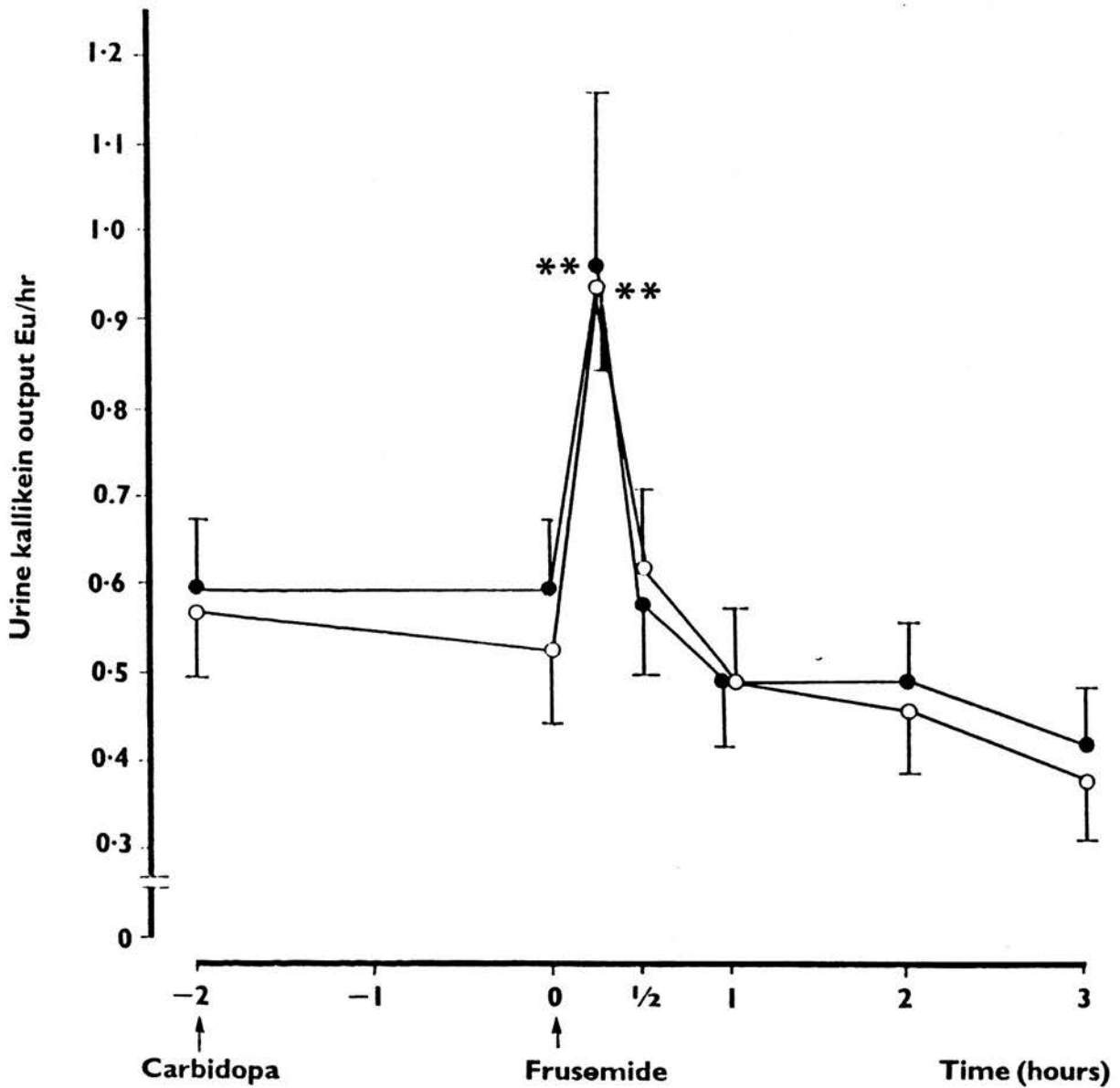


Figure 2c-5. Urine kallikrein output in response to intravenous frusemide (30mg) with (●) or without (○) pretreatment with carbidopa (100mg) in eight subjects. Values are means \pm SEM, ** $p < 0.01$ in comparison with control (0hr).

DISCUSSION

The members of the sulphamoyl benzoate group of drugs, which includes frusemide, bumetanide and piretanide, are described as loop or high ceiling diuretics, and are the most powerful natriuretic drugs in clinical use. Frusemide is actively secreted at the proximal tubule by the organic anion transport system, and inhibits sodium chloride transport from the luminal surface of the nephron. The principle site of action is at the thick ascending portion of the loop of Henlé where it blocks the activity of the Na:K:2Cl cotransporter situated on the luminal membrane (Friedman and Hebert, 1987).

Frusemide is also a renal vasodilator, with increased flow primarily directed towards the deeper renal segments (Data et al., 1978). Glomerular filtration rate shows a less consistent change so that filtration fraction tends to decline (Wilson et al., 1982; Mackay et al., 1984). The demonstration that frusemide reduces proximal tubule fluid and phosphate reabsorption, may be at least partly explained by such alterations in renal haemodynamics (Brenner et al., 1969b; Haas et al., 1977).

The various stimuli to the secretion of renin have been discussed and various groups have focused on the mechanisms by which frusemide and other loop diuretics produce large and sustained increases in renin release. Animal studies, in particular those utilising the non-filtering isolated perfused kidney have shown that the early response to frusemide, that is within 15 minutes, is mediated by intrarenal events with a contribution from both the macula densa and baroreceptor mechanisms (Vander and Carlson, 1969; Corsini et al., 1975). The response to frusemide appears to be biphasic, a secondary rise in renin occurring after one hour or so. Imbs and colleagues (1977) have provided clear evidence, in the anaesthetised dog, that the delayed rise is a function of salt

and water loss, with compensatory activation of the sympathetic nervous system: both propranolol and replacement of urinary losses by ureterovenous anastomosis, though not affecting the immediate rise, abolished the secondary increase in renin release after intravenous frusemide. In agreement with this was a study reported by Cannella et al. (1983) in man, in which significant rises in plasma catecholamines were demonstrated from 60-90 minutes after intravenous frusemide.

The marked natriuretic capacity of both intravenous and oral frusemide (30 mg) was evident in the present studies. After intravenous administration, there was a rapid onset of action with peak response normally within 15 minutes, and drug action was complete by three hours. Concurrent with the natriuresis was a large increase in urine flow. Both inhibition of sodium chloride reabsorption in the thick ascending limb, and an increase in medullary blood flow will lead to reduction in the medullary longitudinal osmolar concentration gradient, impairing normal urinary concentration and leading to the excretion of large volumes of isotonic urine.

The administration of oral frusemide produced a lesser though more prolonged natriuretic and diuretic response, with marked variation between individuals as to the timing of onset and peak responses. This is likely to reflect variable and limited bioavailability, reported to be around 50% in comparison to an intravenous dose (Cutler and Blair, 1979).

The mechanism of action of frusemide, by which a primary natriuretic effect produces a 'solute-diuresis', accounts for the close correlation noted between sodium excretion and urine flow, after both oral and intravenous administration.

In addition to its widespread clinical uses, workers have applied frusemide as a tool to study renal electrolyte handling and in particular, to investig-

ate intrarenal hormonal interactions. Intravenous frusemide, in animals and man, stimulates the production of arachidonic acid and hence the renal synthesis and excretion of a range of prostaglandins including PGE_2 , TXB_2 , $\text{PGF}_{2\alpha}$ and the stable hydrolysis product of prostacyclin, 6-keto- $\text{PGF}_{1\alpha}$ (Weber et al. 1977; Ciabattoni et al., 1979; Wilson et al., 1982; Mackay et al., 1984). In addition, frusemide has been shown to inhibit the prostaglandin degradative enzymes, 15-OH prostaglandin dehydrogenase (Paulsrud and Miller, 1974) and PGE_2 -9-Keto reductase (Stone and Hart, 1976). As already discussed, prostaglandins, particularly PGE_2 and prostacyclin produce, on administration, renal vasodilation and renin release, and in vitro, PGE_2 inhibits sodium reabsorption at the ascending limb of the loop of Henlé. There is ample evidence from studies utilising inhibition of cyclooxygenase, that the prostaglandins largely mediate the renal haemodynamic changes and renin hyperexcretion following intravenous frusemide (Patak et al., 1975; Williamson et al., 1975; Frolich et al., 1976; Weber et al., 1977; Mackay et al., 1984). In the present studies intravenous frusemide produced a rapid and large rise in plasma renin activity, with levels thereafter declining but remaining significantly elevated above baseline three hours later. The prostaglandins act as modulators to known stimuli for renin release, but do also provide an independent separate input to the juxtaglomerular cells. The marked prostaglandin-dependence of the renin response following intravenous frusemide was demonstrated by the abolishment of both the early and sustained rise in plasma renin activity after the prior administration of indomethacin. A dose of 100 mg of indomethacin is known to decrease the urinary excretion of PGE_2 by 80% within one hour, with the effect persisting for at least 4 hours (Mackay et al., 1984).

It is of note that the renin response was distinct following oral frusemide. There was no early rise and levels gradually increased over five hours, a

phenomenon presumably reflecting the progressive, cumulative, salt and water deficit.

In the present studies, indomethacin did not apparently affect baseline sodium excretion. However the peak natriuretic response was smaller, and total sodium excretion over 3 hours, in response to frusemide, was significantly and appreciably attenuated by the order of 25% ($p < 0.01$) in the presence of indomethacin.

There is less consensus in the literature on the importance of prostaglandins in the natriuretic capacity of frusemide. Conflicting reports probably reflect differences in protocol, animal model, and experimental technique. In addition, chronic dosing studies are complicated on account of renal compensatory adjustment that may occur. An important factor appears to be the pre-existing state of the renal circulation, such that under conditions of antinatriuresis and increased vascular tone, the prostaglandins assume an important controlling function in modulating the effects of activated renin-angiotensin and sympathetic nervous systems (reviewed by Dunn and Zambraki, 1980; Patrono and Dunn, 1987). Thus Berg (1977) showed in humans with renal impairment, that acetyl salicylic acid decreased the natriuresis produced by an intravenous infusion of frusemide. Similarly, in cirrhosis (Planas et al., 1983) and the nephrotic syndrome (Tiggeler et al., 1977), both conditions characterised by intravenous volume depletion, the natriuretic response was markedly inhibited by cyclooxygenase inhibition.

In normal man and animals, inhibition of prostaglandin synthesis has been demonstrated either to significantly attenuate, but never abolish, the natriuretic response to frusemide (Patak et al., 1975; Frolich et al., 1976; Brater, 1979; Mackay, 1984), or produce no change (Bailie et al., 1976; Weber et al., 1977). In a study performed in dogs, Neis et al. (1983) argued that the

reduction in natriuresis is due to blockade of the renal haemodynamic effects: in salt depleted dogs, indomethacin completely blocked the increment in renal blood flow and decreased the peak sodium excretion produced by frusemide. In contrast, in sodium replete dogs, frusemide did not increase renal blood flow, and indomethacin had no effect on either renal haemodynamics or natriuresis. On a rather similar vein, Brater et al. (1979) noted a reduction in the natriuretic response to 40 mg of intravenous frusemide in man, but no difference in fractional excretion of sodium after normalising for a small decline in creatinine clearance, in the presence of indomethacin.

Indomethacin has been reported to have a large number of actions in addition to prostaglandin-inhibition (Dunn and Zambraski, 1980) and the authors caution against presuming that any physiological function is prostaglandin mediated if it is attenuated after indomethacin. In addition, indomethacin, like other non-steroidal anti-inflammatory drugs, is secreted through the proximal tubule by the same organic anion transport system utilised by frusemide, thus presenting a possible pharmacokinetic interaction, distinct from any effect on prostaglandins. In a study in man, indomethacin did not affect the total amount nor the time course of the urinary excretion of frusemide. However, a pharmacodynamic interaction was evident in that the dose-response curve, relating urinary frusemide excretion rate to sodium excretion rate, was shifted downwards and to the right (Chennavasin et al., 1980). Further support for a direct tubular interaction comes from an in vitro study demonstrating that prostaglandin inhibition alters chloride uptake at the loop of Henlé after frusemide administration (Kirchner, 1985).

Whether the attenuation of natriuresis by indomethacin in the present study was related to haemodynamic or tubular events cannot be answered. However in a similar study in salt replete subjects, Mackay et al. (1984)

demonstrated that indomethacin blocked the frusemide-induced increment in renal blood flow associated with a significant reduction in sodium excretion.

By means of salt loading manoeuvres, various groups have claimed to have identified subsets of essential hypertensives which are particularly sensitive, in terms of blood pressure responses, to the effect of an increase in dietary salt (Fujita et al., 1980; Weinberger et al., 1986). Harvey et al. (1984) showed in a small group of essential hypertensives, that dopamine mobilisation in the kidney is defective in the face of a salt load. Shikuma and colleagues (1986) extended this work, and showed that only subjects characterised as being 'salt-sensitive' hypertensives possessed the abnormal dopamine response. They postulated that the lack of appropriate rise in dopamine on salt loading might prevent normal sodium excretion, and predispose individuals to salt retention and hypertension. The demonstration of 'salt-sensitivity', by salt loading over a number of days, is laborious and not easily widely applicable. Kuchel and colleagues (1979) had made the interesting observation that intravenous frusemide, produced an acute rise in urine dopamine excretion. In addition, both the dopamine and natriuretic response was impaired in essential hypertension. However, the group did not clearly characterise the response in terms of sodium and volume output, or the relationship to other hormonal systems in the kidney, and the mechanism was not considered. It was of interest to follow up this observation to determine whether the dopamine response to frusemide is consistent in normal subjects. Further studies, beyond the scope of the present work, might then assess the response in essential hypertension, with a particular aim of identifying salt-sensitive patients. The use of frusemide as a simple test in this way would be rather analagous to its use in the separation of renin-responsive subsets in essential hypertension (Padfield et al., 1975).

In this department, a preliminary study had demonstrated an actual decline in urine dopamine excretion after oral frusemide. The present work therefore sought to further characterise the dopamine response after oral administration and allow comparison with changes after intravenous frusemide in the same subjects. After oral frusemide there was a small increase in dopamine excretion within 15 minutes of administration, occurring in all subjects. Thereafter, levels declined back to baseline and at 4 hours a significant decline from control was apparent. The explanation for the early increase is not straightforward. Although sodium output and urine flow had started to increase in all volunteers by this time, peak responses did not occur for a further 1-2 hours, and relatively little drug would have been expected to be absorbed at this stage. The decline in dopamine output may be a feature of the induced salt and water depletion, but such a pattern was not seen after intravenous frusemide in spite of greater cumulative natriuresis. Further studies should be performed with different doses of oral frusemide to determine dopamine responsiveness. However at 30 mg, overall changes were small and, due to limited bioavailability and variable interval to peak natriuresis, it seems unlikely that oral administration of frusemide will provide a standard and reproducible method for stimulating appreciable dopamine release.

The results confirm that intravenous frusemide produces a prompt, and significant increase in urine dopamine excretion. The peak response at 15 minutes represented at least a doubling of dopamine output before a steady decline and return to baseline around $1\frac{1}{2}$ hours. After intravenous frusemide, the rise in urine dopamine excretion correlated closely with that of both natriuresis and urine flow rate, although it was noticeable that dopamine excretion had declined at a time when sodium output was still appreciable, and

the latter did not reach control values until 3 hours after dosing. The excretion of both prostaglandins and kallikrein can, under certain conditions, be demonstrated to be flow dependent. One possibility is the washout of preformed hormone from the tubular lumen. It is conceivable that the increase in dopamine also represents a "washout" phenomenon. However when normal subjects undertook 24 hour urine collections, urine dopamine excretion correlated closely with sodium output, but not with urine flow rate (Casson, 1984). Also, under the baseline condition of this study, urine dopamine excretion appeared to have a closer relationship to sodium excretion than to flow rate. It has also been shown in dogs, that intravenous volume loading with saline increases dopamine output, but that similar expansion with albumin solution does not (Faucheux et al., 1977), pointing to the importance of the sodium (or chloride) ion in dopamine stimulation. Thus prevailing evidence might suggest that the dopamine response after frusemide is causally related to the natriuresis (*vide infra*).

Pretreatment of subjects with indomethacin did not alter the output of dopamine in the baseline state, either in the seven subjects compared just before frusemide was given, or in the three subjects studied over five hours. In addition, in spite of a 25% reduction in the natriuretic response to frusemide, indomethacin did not alter the pattern of dopamine release after frusemide, either in magnitude or time course. The results suggest that the dopamine response to frusemide, in contradistinction to other effects of the diuretic, is not dependant on the availability of prostaglandins. As previously described, the dose of indomethacin used would be expected to markedly impair prostaglandin synthesis. It remains possible that residual prostaglandin synthesis, resistant to the action of indomethacin, could have mediated the increase in dopamine, but the completeness of inhibition of the renin response, as an

indicator of active prostaglandin synthesis, renders this unlikely. This conclusion has implications for the possible mechanisms by which frusemide stimulates dopamine excretion. The limiting factor, under normal circumstances, for dopamine synthesis in the proximal tubular cell may be the availability of L-dopa, and the uptake of this compound into tubular cells appears to be dependent on the sodium chloride concentration within the tubular lumen (Ullrich et al., 1974). Frusemide has inconsistent effects on the filtered load of sodium, but renal vasodilatation, by altering the peritubular physical forces, will ensure a relatively increased sodium load at the proximal tubule. It could then be postulated that it is the change in renal haemodynamics that modulates dopamine synthesis. Renal haemodynamic monitoring was not carried out in this work, but in the study already referred to (Mackay et al., 1984), indomethacin prevented the rise in renal blood flow. As a result, a reduced dopamine response would have been expected in the present study. This was not the case however, and it would seem necessary to propose another mechanism for dopamine excretion after pharmacological stimulation with frusemide. There is at present no evidence that the dopamine synthetic process can respond to an increase in salt load at a more distal site in the nephron, though such a mechanism cannot be discounted. An alternative unproven possibility is that frusemide directly stimulates dopamine production at the proximal tubule. The drug is directly secreted through proximal tubular cells, the site of active dopamine synthesis, and as discussed, indomethacin does not interfere significantly with its transport into the urine. In addition, the secretion rate of frusemide correlates closely with the natriuretic response (Cutler and Blair, 1979). This question deserves further study and might be approachable with *in vitro* tubular preparations.

From the reported literature and the results of the present studies, the

prostaglandins appear to mediate only a small proportion of the natriuretic response to frusemide. Dopamine, when administered exogenously, produces renal vasodilation and an increase in sodium excretion, effects similar to those exerted by frusemide. It was therefore of interest to determine whether the acute rise in endogenous dopamine, produced in response to frusemide, contributed in any way to the renal effects of the diuretic. Carbidopa in a single dose of 100 mg by mouth, markedly inhibited dopamine excretion during the baseline period, the effect being apparent by one hour, and levels being unrecordable within two hours. Thereafter, the rise following frusemide was abolished and levels remained below the limit of detection for a further three hours. Baseline natriuresis was not altered by the presence of carbidopa, and it is clear that dopamine synthesis blockade with carbidopa did not significantly affect the natriuretic response to frusemide, either in magnitude or time course. Therefore, under the conditions of these studies, dopamine did not contribute to the natriuretic action of frusemide and its enhanced excretion must be regarded as an epiphenomenon. Whether the dopamine response to intravenous frusemide, could provide a discriminatory test in essential hypertension remains to be determined. The mechanism of the increase in dopamine excretion is still unclear, and may not relate directly to salt handling by the kidney. Whether or not this is so, a pharmacological stimulus, such as that produced by frusemide could still provide an indication of the capacity of an individual to mobilise dopamine, which may relate to the physiological stimulus of salt loading.

The potential interactions of renin and dopamine in the kidney have already been discussed in some detail. The studies with frusemide allowed analysis of the relationship from two approaches. Firstly, prostaglandin-synthetase inhibition with indomethacin tended to lower plasma renin activity in

the baseline state and abolish the rise induced by frusemide, but did not affect the output of dopamine. Secondly, almost complete inhibition of dopamine synthesis did not alter plasma renin activity, either at baseline or after frusemide. Therefore, dopamine is not important in the renin response to frusemide which is markedly prostaglandin-dependant, and conversely, changes in renin release do not appear to alter the output of dopamine.

An acute increase in urine kallikrein and kinin excretion has previously been reported in man after intravenous frusemide (Abe et al., 1978), and this was confirmed in this work for kallikrein output. The rise was short-lived and levels had returned to baseline by 30 minutes after administration. It is clear, that dopamine plays no part in the kallikrein response after such pharmacological stimulation: the pattern of excretion after dopamine blockade with carbidopa was almost identical to that in the presence of placebo.

Further light may be shed upon the dopamine response to frusemide by study of other diuretics with actions on different segments of the nephron. Although there is doubt as to whether the thiazide diuretics stimulate prostaglandin release (Patak et al., 1979; Kramer et al., 1980), they do not appear to alter renal blood flow or renin release in the acute situation (Brater, 1986; Imbs et al., 1977). It would therefore be of interest to determine the dopamine response to such drugs which, exert a primary natriuretic action on the distal tubule, but do not influence renal haemodynamics.

Frusemide, after intravenous administration, produced an acute rise in mean arterial pressure at 15 minutes, with values declining thereafter. The diuretic is known to exert extra-renal haemodynamic effects, producing systemic venodilatation and reduction in left ventricular filling pressure (Dikshit et al., 1974; Johnston et al., 1983a). In addition, in humans, it produces an early reduction in forearm blood flow with arterial

vasoconstriction. Such effects are apparent within 15 minutes of administration and are therefore distinct from diuresis and reduction in extracellular fluid volume. The extrarenal effects of frusemide depend on the presence of functioning kidneys (Bourland et al., 1977) and are abolished in the presence of both cyclooxygenase inhibition and angiotensin converting enzyme inhibition (Johnston et al., 1983a,b). Johnston's group has therefore proposed that renin, released from the kidney, is the principle mediator. In the peripheral circulation, stimulated angiotensin II produces arterial vasoconstriction, whereas at the vein wall it promotes the release of dilatory prostaglandins. The present results are compatible with such a hypothesis: in the presence of indomethacin, frusemide did not increase blood pressure, presumably due to the inhibition of renin release. Blood pressure was however elevated prior to the administration of frusemide and remained elevated above values obtained with placebo. That cyclooxygenase inhibition is hypertensive is widely accepted (Carmichael and Shankel, 1985). The propensity for indomethacin to produce sodium retention cannot explain the increase in blood pressure within 1½ hours of administration. It is more likely that inhibition of extrarenal cyclooxygenase and decrease in vascular wall prostaglandin synthesis allows vasoconstrictor influences to act unopposed, leading to increased resistance of systemic vascular beds. In this context, there is evidence that the inhibition of prostaglandin synthesis may increase sympathetic nerve activity (Samuelsson and Wennmalm, 1971). The haematocrit data give further information on systemic responses induced by frusemide. After frusemide, haematocrit increased significantly from 15 minutes and then remained elevated throughout the study. Such an early rise cannot be explained on the basis of extracellular fluid loss, and the mechanism is unknown but must reflect extra-renal actions of the drug. It may be due to increased capillary transudation resulting from

altered arterial and venous tone, or the drug may directly alter red cell size. This interesting observation requires further study.

Whether dopamine receptors on the systemic vasculature normally have a modulating role in systemic vascular resistance and blood pressure seems, as discussed, unlikely. Carbidopa did not influence blood pressure in the baseline state, but at one point after frusemide there was an elevation, compared to placebo, though the rise was not sustained. The significance of this isolated result is unclear.

In summary, the renal effects of frusemide are markedly influenced by prostaglandin-synthesis inhibition. However, the acute increase in dopamine excretion is not mediated through the prostaglandins and no interaction between the hormonal systems is apparent. In addition, dopamine does not contribute to the action of frusemide in the kidney.

CHAPTER FIVE

THE EFFECT OF CARBIDOPA AND LITHIUM ON THE SYSTEMIC AND RENAL RESPONSE TO ACUTE INTRAVENOUS SALINE LOADING IN NORMAL MAN

INTRODUCTION

Expansion of the ECF space by intravenous saline loading provides an effective stimulus to sodium excretion. Much work has been performed in animals and man to elucidate the mechanisms involved. De Wardener and colleagues (1961) demonstrated in dogs that a natriuretic response to volume expansion persisted in the absence of changes in RBF, GFR and mineralocorticoid activity. This led to the search for other possible natriuretic factors. One such factor is endogenous dopamine. Studies in animals and man have confirmed that an acute salt load leads to an increase in urinary excretion of dopamine (Faucheux et al., 1979; McClanahan et al., 1985; Alexander et al., 1974). With the known natriuretic effect of exogenous dopamine, it was of interest to determine whether dopamine is a contributory factor in the natriuretic response. It was demonstrated in a previous chapter that the dopa decarboxylase inhibitor, carbidopa, markedly reduced urinary dopamine output. In the present study, therefore, the effect of carbidopa on renal and systemic responses to saline infusion was assessed in normal subjects, to determine any possible dopaminergic component.

Measurement of the renal clearance of lithium has been used to investigate the tubular nephron segments involved in salt excretion. Lithium carbonate was therefore administered to determine those segments contributing to the natriuresis of intravenous saline loading. In a previous chapter, the potential pitfalls of its use as a marker of renal function were highlighted. In particular, it elevated sodium excretion but subsequently attenuated the natriuresis produced by gludopa. It was important to determine whether lithium affected the natriuretic response to the volume expansion, either through influencing the dopaminergic, or some other mechanism. Lithium was therefore

given on a separate day in the presence of intravenous saline, to assess the effect of the ion per se, and a larger dose of 1000 mg was chosen to accentuate any possible interaction.

Protocol

Nine subjects were each studied on three occasions. After a 2 hour run-in (0-2) period, subjects received an intravenous infusion of 0.9% isotonic saline (20 mls/kg/hr) over 3 hours (2-5) followed by a 2 hour recovery period (5-7). At the end of the 7th hour, a light snack was provided which contained almost no protein and no more than 3 mmol of sodium. Thereafter, they were allowed to rise, and urine, passed at will, was collected for a further 4 hours (7-11). Each subject took on separate occasions, either no active drug, carbido-*opa* 100 mg by mouth at 0 and 5 hours, or lithium carbonate, 1000 mg by mouth, at 2200 hours on the evening before study. Placebo tablets were used such that tablets were taken every dosing time. At each hour (1-7), supine blood sampling, and blood pressure and pulse measurements were followed by voiding and collection of urine samples. For analysis, the control baseline results were mean values for the 2 hour run-in period.

Results

None of the subjects felt unwell during the study manoeuvres. Some however, noted swelling of the fingers and two experienced a change in voice quality during saline infusion.

Renal and systemic response to intravenous saline infusion

In the presence of placebo, sodium excretion rose steadily during intravenous volume expansion from 0.15 ± 0.03 mmol/min during the run-in period to

reach a peak of 0.73 ± 0.12 mmol/min in the hour following the infusion ($p < 0.01$, Figure 3a-1), representing a five-fold increase. Levels declined after this point but remained well above control during the final four-hour urine collection. The degree of intravenous volume expansion was evident from the sharp drop in haematocrit (41.9 ± 0.9 to $36.4 \pm 0.8\%$, $p < 0.01$) during the last hour of infusion (Table 3a-1). During the entire study only about 26% of the administered salt load was excreted. This was reflected in the persistent rise in body weight in all subjects.

Salt loading produced a large fall in PRA, the greatest change being evident during the first hour (2.06 ± 0.28 to 0.92 ± 0.16 ng angiotensin I ml⁻¹ h⁻¹, $p < 0.01$, Figure 3a-2). Plasma ANP exhibited only a modest rise during the first hour of infusion which was not significant. By the last hour of infusion, however, levels had increased to 240% of control values (42.7 ± 6.0 to 103.0 ± 10.2 pg/ml, $p < 0.01$, Figure 3a-3). ANP then tended to decline, though it remained significantly above control two hours after the end of the infusion. During the period of intravenous saline infusion, urine dopamine excretion did not change from control values (Figure 3a-4). A modest but significant rise of about 26% was apparent during the hour after the infusion (1.33 ± 0.12 to 1.67 ± 0.13 nmol/min, $p < 0.05$), and this was maintained at the end of the study.

Creatinine clearance tended to decline during saline infusion but there were no significant changes throughout the study (Table 3a-2). The systemic parameters, mean arterial pressure and pulse were unchanged.

The effect of carbidopa on responses to intravenous saline infusion

Carbidopa, administered orally (100 mg) at the start of the study (0 hr) and again at 5 hours, effectively blocked dopamine excretion during the 11 hours of the study. In seven of the subjects, levels were markedly depressed

towards the limit of detection of the HPLC assay. Mean values shown in Figure 3a-4 were elevated by values in two subjects in whom the levels fell only modestly in response to carbidopa. By the final collection period, dopamine excretion had started to rise, presumably reflecting recovery from dopa decarboxylase inhibition. It is evident from the results that blockade of dopamine synthesis did not affect renal and systemic parameters measured during the control period. In addition, sodium excretion was comparable at all time points during and after the infusion of saline, the response curves being almost superimposed (Figure 3a-1). The responses of PRA (Figure 3a-2), creatinine clearance (Table 3a-2), mean arterial pressure and pulse (Table 3a-1) were similar in the presence of either placebo or carbidopa. Plasma ANP levels tended to be lower after pre-treatment with carbidopa, but there were no significant changes in comparison with placebo during volume expansion.

The effect of lithium on responses to intravenous saline infusion

Lithium carbonate, administered in a dose of 1000 mg by mouth on the evening before the study, resulted in a mean serum lithium level of 0.40 ± 0.03 mmol/l 11 hours later at the start of the study. Analysis of the two hour run-in period revealed that in the presence of lithium, baseline sodium excretion was appreciably elevated compared to values obtained on placebo (0.21 ± 0.04 vs 0.15 ± 0.03 mmol/min) though the change just failed to reach significance by students' t-test ($p < 0.08$). Lithium resulted in a significant increase in PRA at baseline (2.80 ± 0.24 vs 2.06 ± 0.28 ng angiotensin I $\text{ml}^{-1} \text{h}^{-1}$, $p < 0.01$).

During intravenous volume expansion, sodium excretion increased significantly. However, after one hour, the excretion rate at each point tended to be lower when compared with placebo, and the peak natriuretic response was less than three times that of the control value (0.21 ± 0.04 to 0.58 ± 0.06

mmol/min, Figure 3a-5) in the hour after the infusion. The absolute increment in sodium excretion above the control values was lower at all time points and cumulative natriuresis compared with control was reduced significantly by lithium (106.8 ± 30.1 vs 179.6 ± 29.1 mmol) representing about a 40% reduction (Figure 3a-6). PRA declined sharply during saline infusion to values similar to those on placebo alone (Figure 3a-2). Plasma ANP and urine dopamine were similar on lithium and placebo during the baseline period and exhibited comparable increments during saline loading (Figures 3a-3, 4). The responses of mean arterial pressure, pulse and creatinine clearance were not significantly changed by lithium (Tables 3a-1, 2). Lithium clearance data are depicted in Table 3a-3. There was a highly significant increment in lithium clearance both during the period of saline infusion and during the succeeding two hour recovery period ($p < 0.01$). Similarly, lithium clearance expressed as a fraction of creatinine clearance increased significantly. The ratio of sodium to lithium clearance, representing the percentage of fluid escaping reabsorption in the "distal tubule", increased, and was significant during the recovery period ($p < 0.01$). In spite of this, absolute "distal tubular" sodium reabsorption increased markedly during both periods in comparison with control ($p < 0.01$).

	Time (hours)					
	C	3	4	5	6	7
Placebo						
Mean arterial pressure (mm Hg)	85.4 ± 3.3	86.4 ± 2.8	88.3 ± 3.2	88.4 ± 3.4	86.6 ± 3.2	88.5 ± 3.2
Mean pulse rate (beats/min)	57 ± 2	55 ± 2	56 ± 3	61 ± 3	59 ± 3	59 ± 3
Haematocrit (%)	41.9 ± 0.9	38.4** ± 0.5	-	36.4** ± 0.8	-	36.9** ± 0.8
Body weight (Kg)	79.9 ± 6.3	82.4** ± 6.8	-	83.3** ± 6.5	-	82.6** ± 6.4
Carbidopa						
Mean arterial pressure (mm Hg)	88.6 ± 3.1	86.9 ± 3.3	90.1 ± 3.9	91.4 ± 4.6	88.1 ± 3.4	88.0 ± 3.5
Mean pulse rate (beats/min)	59 ± 2	56 ± 2	58 ± 3	61 ± 4	62 ± 3	60 ± 3
Haematocrit (%)	41.9 ± 0.9	39.0** ± 1.1	-	37.1** ± 1.1	-	37.6** ± 1.0
Body weight (Kg)	79.9 ± 6.4	80.9** ± 6.4	-	83.0** ± 6.5	-	82.3** ± 6.4
Lithium						
Mean arterial pressure (mm Hg)	87.5 ± 3.2	86.8 ± 3.1	87.8 ± 3.6	91.6 ± 4.5	91.3 ± 4.4	93.6 ± 4.1
Mean pulse rate (beats/min)	58 ± 2	56 ± 3	59 ± 3	58 ± 4	56 ± 2	57 ± 3
Haematocrit (%)	43.0 ± 1.0	39.8** ± 0.8	-	37.7** ± 0.9	-	38.9** ± 1.0
Body weight (Kg)	78.9 ± 6.3	80.1** ± 6.3	-	82.3** ± 6.3	-	81.7** ± 6.4

Table 3A-1

Effect of intravenous saline (infused during hours 3, 4 and 5) on systemic parameters in nine subjects. Results are expressed as means ± SEM; *p<0.05; **p<0.01 in comparison with control.

	Control (hours 0-2)	Saline infusion (hours 2-5)	Recovery (hours 5-7)
Placebo	131.5 + 8.2	123.7 + 10.0	145.6 + 11.1
Carbidopa	146.2 + 9.7	140.9 + 13.1	142.3 + 9.7
Lithium	123.5 + 4.9	115.4 + 3.6	126.9 + 7.0

Table 3A-2 Effect of intravenous saline on creatinine clearance in nine subjects. Results are expressed as means \pm SEM.

	Control (hours 0-2)	Saline (hours 2-5)	Recovery (hours 5-7)
Lithium clearance (mls/min)	27.0 ± 2.3	34.5* ± 4.3	37.0** ± 5.0
Fractional lithium clearance (%)	22.3 ± 1.7	30.1** ± 3.5	28.9* ± 3.0
Fractional distal tubular sodium clearance (%)	5.9 ± 1.1	8.6 ± 1.2	11.1** ± 0.8
Absolute distal tubular sodium reabsorption (mmol/min)	3491 ± 304	4403* ± 560	4591** ± 650

Table 3A-3 Effects of intravenous saline on tubular function in nine subjects. Results are expressed as means ± SEM; * p<0.05, ** p<0.01 in comparison with control.

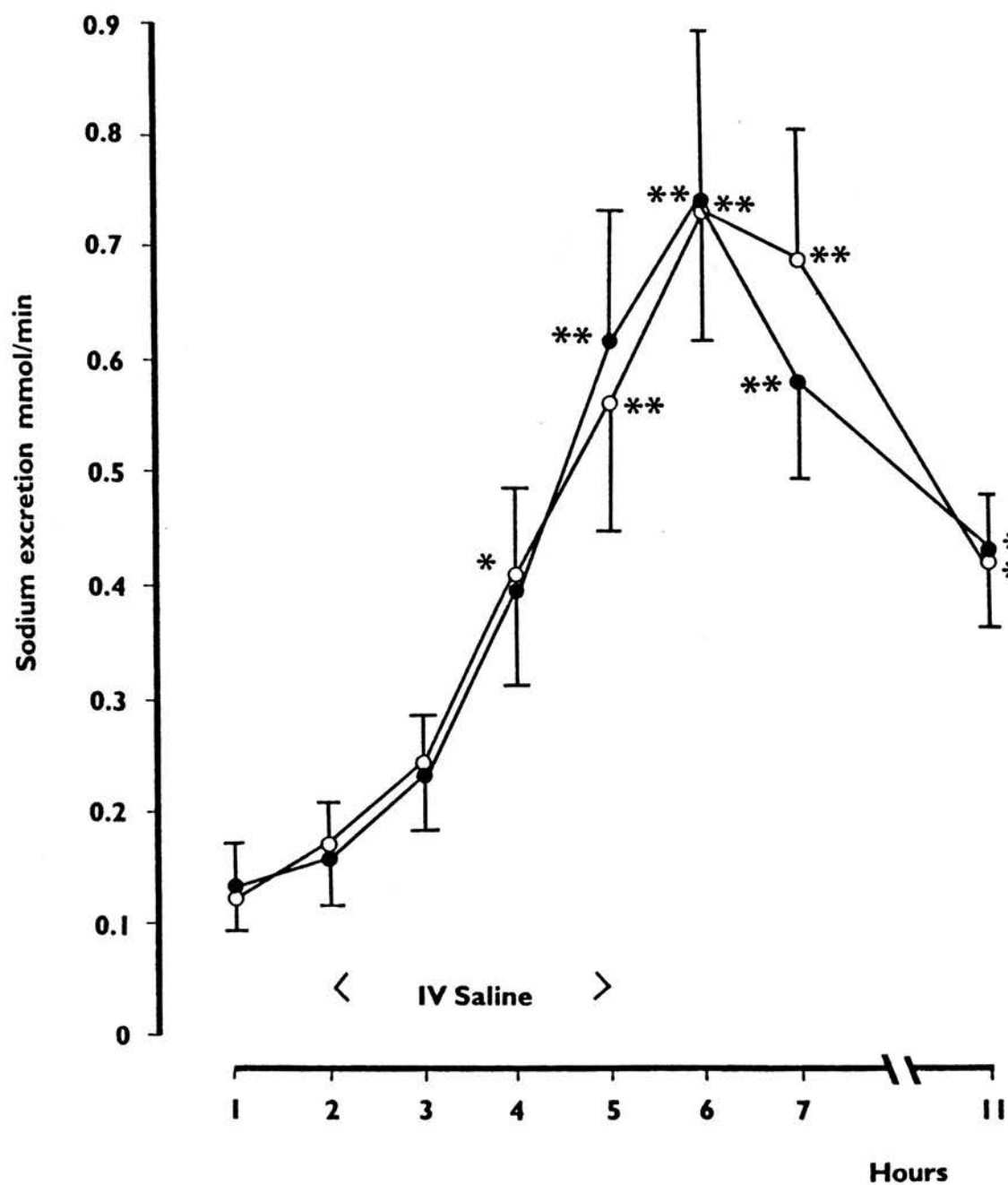


Figure 3a-I. Urine sodium excretion in response to intravenous saline loading in nine subjects pretreated with placebo (○), or carbidopa (●). Values are means \pm SEM; * $p < 0.05$, ** $p < 0.01$ for comparison with control.

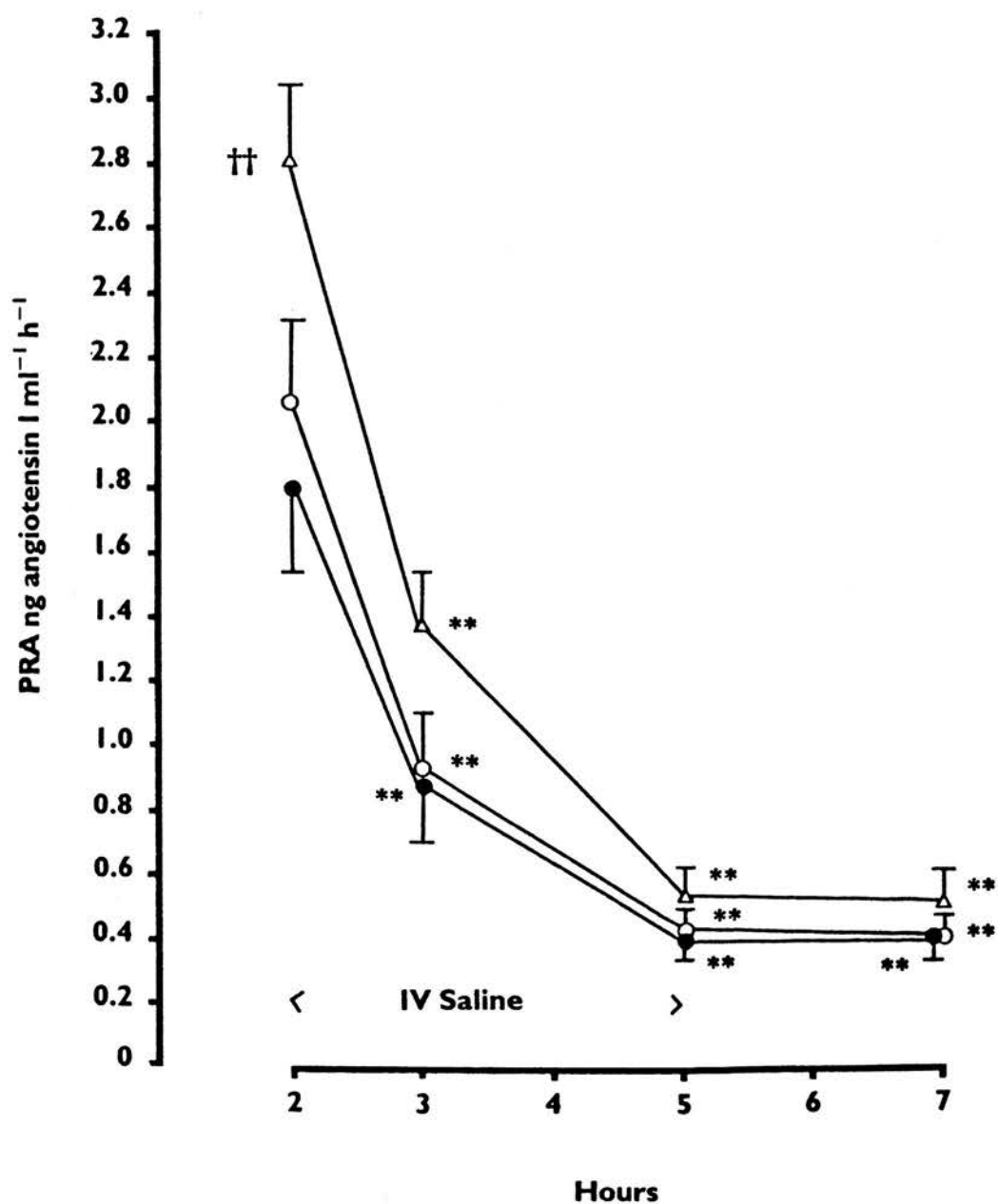


Figure 3a-2. Plasma renin activity in response to intravenous saline loading in nine subjects pretreated with placebo (○), lithium carbonate (△), and carbidopa (●). Values are means \pm SEM; ** $p < 0.01$ for comparison with control. $\dagger\dagger$ $p < 0.01$ for comparison between lithium and placebo

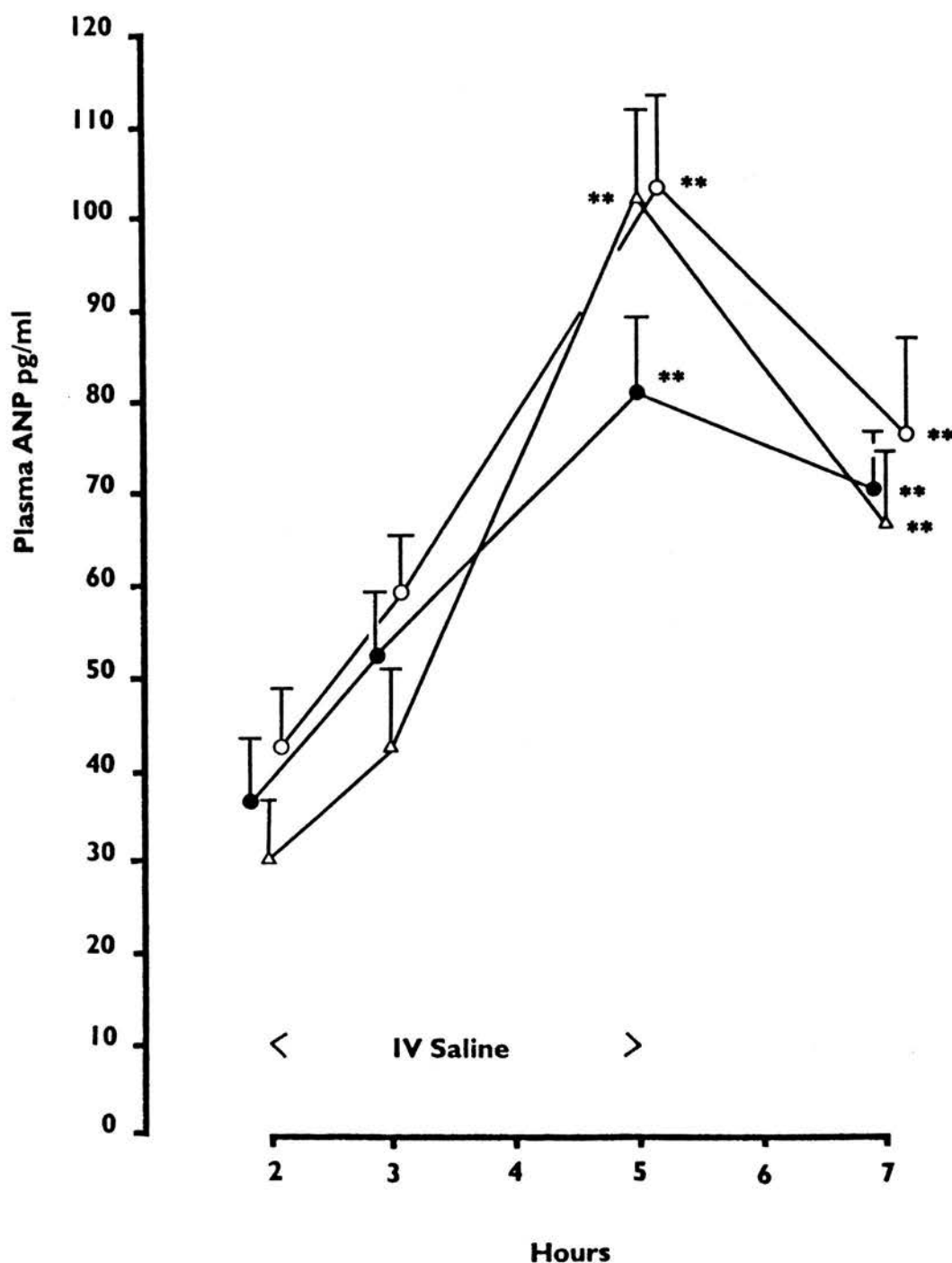


Figure 3a-3. Plasma ANP in response to intravenous saline loading in nine subjects pretreated with placebo (○), lithium carbonate (△), and carbidopa (●). Values are means \pm SEM; ** $p < 0.01$ for comparison with control.

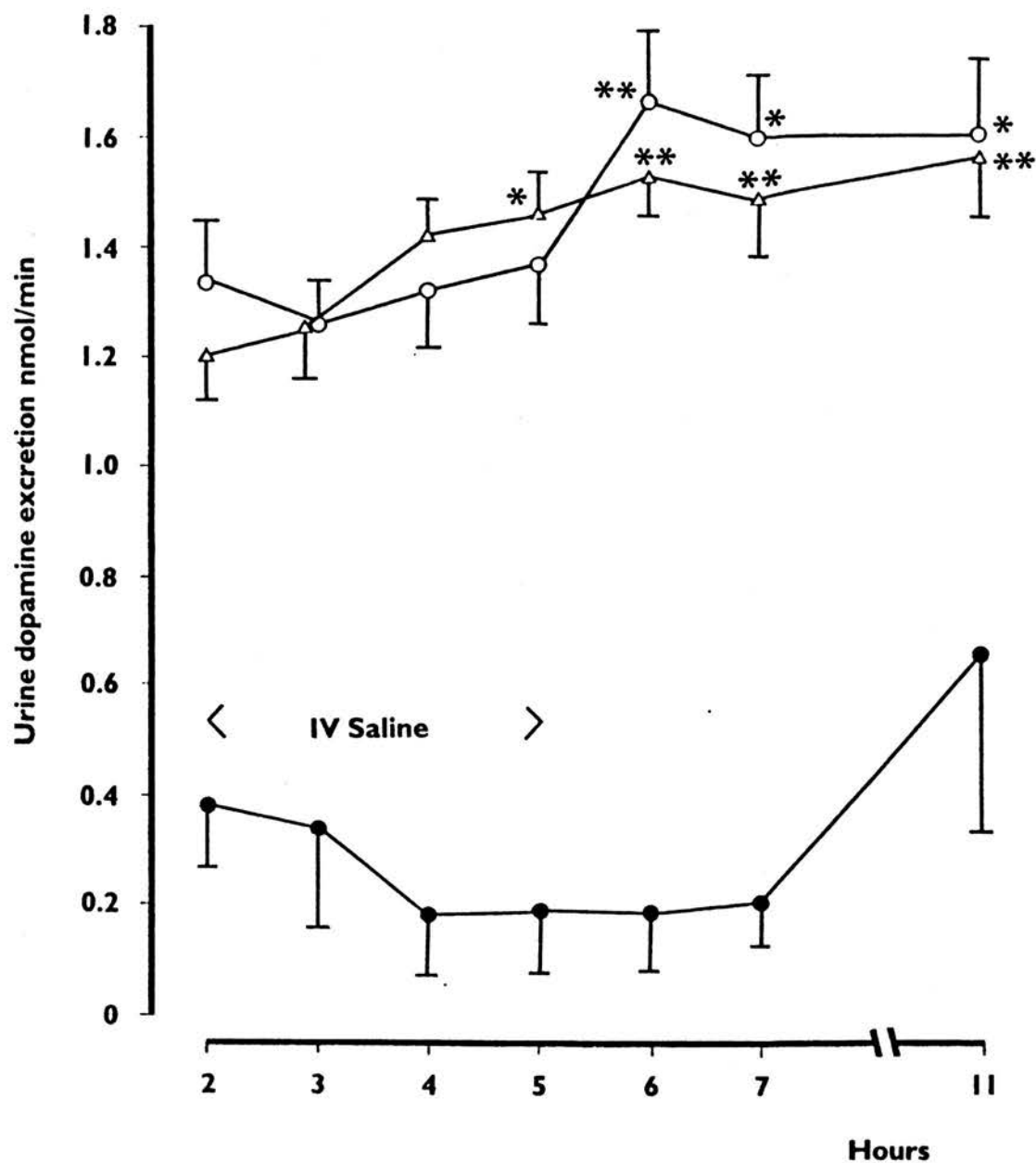


Figure 3a-4. Urine dopamine excretion in response to intravenous saline loading in nine subjects pretreated with placebo (○), lithium carbonate (△), and carbidopa (●). Values are means \pm SEM; * $p < 0.05$, ** $p < 0.01$ for comparison with control.

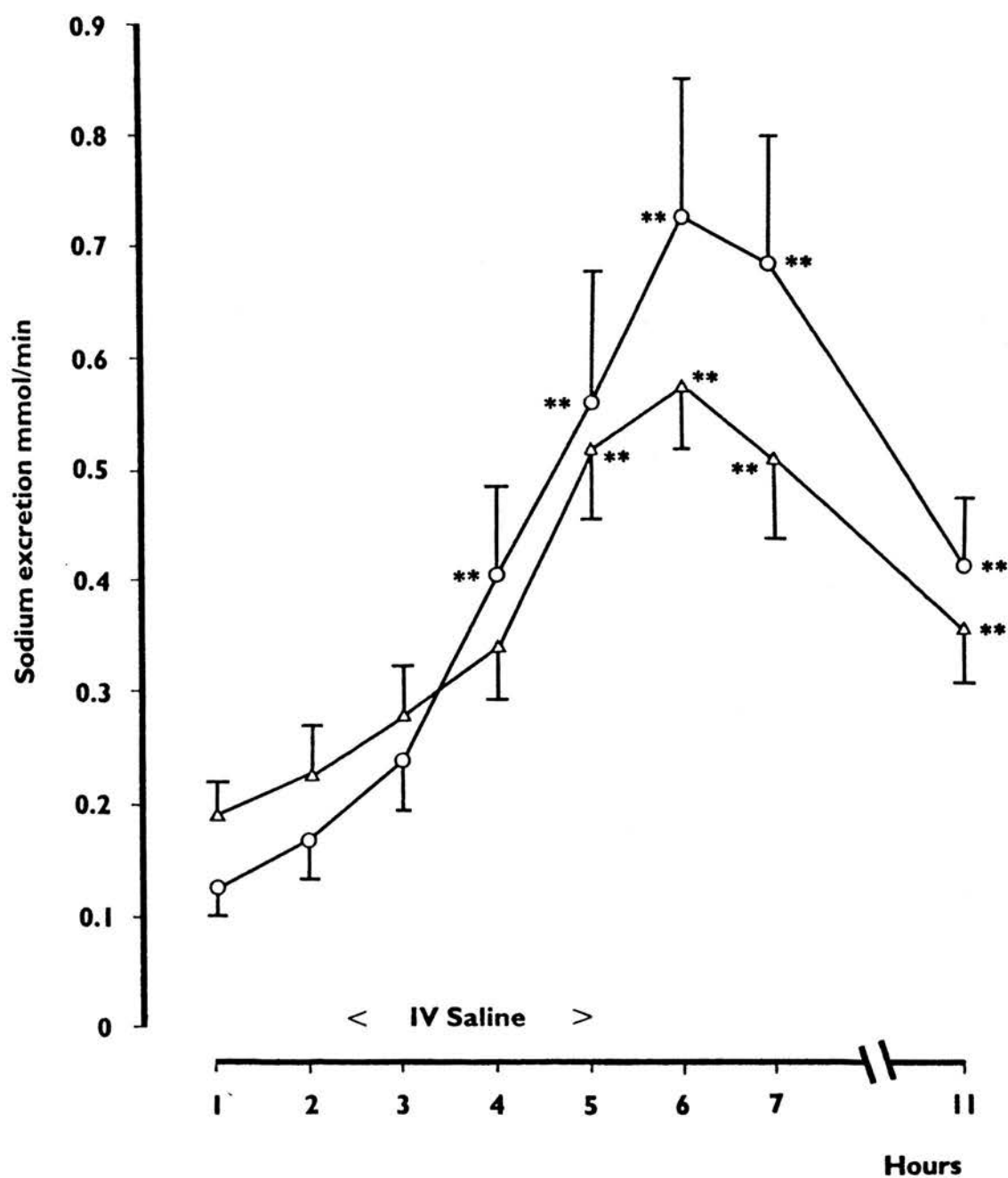


Figure 3a-5. Urine sodium excretion in response to intravenous saline in nine subjects pretreated with placebo (○), or lithium carbonate (△). Values are means \pm SEM; ** $p < 0.01$ for comparison with control.

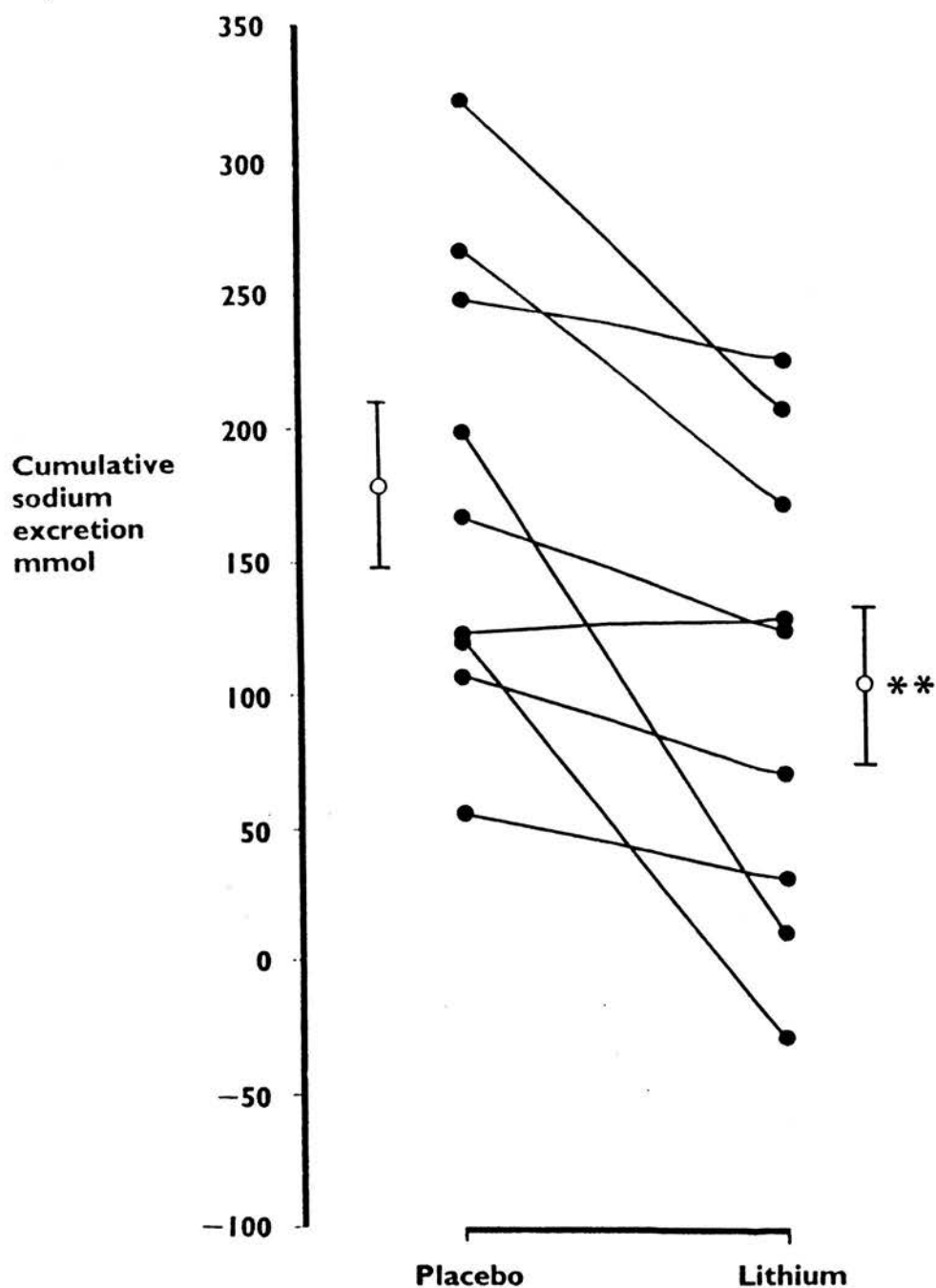


Figure 3a-6. Cumulative sodium excretion above control over 9 hours in response to intravenous saline loading after pretreatment with placebo or lithium carbonate. Individual values and means \pm SEM are plotted for nine subjects; ** $p < 0.01$.

DISCUSSION

Expansion of the circulating blood and ECF volume has been widely used as a method to analyse mechanisms of natriuresis. The most widely used technique is the administration of an acute intravenous load of isotonic saline. It should be noted that such a manoeuvre is likely to provide a supra-physiological stimulus, but by placing a stress on the system, it allows determination of those factors which can modulate sodium excretion. Similar expansion of the effective circulating volume can also be achieved by water immersion and lower body positive pressure applied by inflating cuff (Coruzzi et al., 1986; Bennett et al., 1982).

In this study, the infusion of saline at a rate of 20 ml/kg each hour resulted, in normal volunteers, in a total load of about 4.5 litres over 3 hours. The effectiveness of expansion of the vascular space was evident from the marked drop in haematocrit values. During the placebo infusion sodium excretion started to increase during the first hour, and the peak response, representing a 5-fold rise above baseline, occurred during the first hour after the infusion. Excretion was still elevated during the final 4-hour collection period, though levels were starting to decline. In spite of the marked natriuretic response, only around 26% of the administered sodium was excreted in the 9 hours of study and this was reflected in the persisting increment in weight. To encompass the total response in sodium excretion would have required a much more prolonged study which was not feasible.

The components of the natriuretic response following saline infusion have attracted great interest and possible factors were discussed in the introductory chapter. Sodium excretion represents the final outcome of the balance of opposing natriuretic and anti-natriuretic influences. Volume expansion with

saline is associated with reduced activity of the sympathetic nervous system, with reduction in urinary noradrenaline excretion (Alexander et al., 1974), and inhibition of the renin-angiotensin-aldosterone axis. A reduction of tonic influence of these systems would be expected to promote natriuresis. Certainly a marked and sustained reduction in PRA was evident in this study, the largest decrement occurring during the first hour of infusion.

Other influences may also play a facilitatory role. The renal prostaglandin and kallikrein-kinin systems, and the Na^+/K^+ ATPase inhibitor have been advocated as important influences in the response to saline, but their role was not determined in the present study. Although increases in systemic blood pressure and GFR may be associated with increased salt excretion, it has been well demonstrated that such changes are not mandatory or limiting (Krishna et al., 1985; de Wardener et al., 1961), and these parameters were unchanged in this study in spite of the marked response. Similarly, although inhibition of aldosterone release produced by volume expansion removes an antinatriuretic influence, natriuresis persists in the presence of mineralocorticoid excess (de Wardener et al., 1961).

Micropuncture studies have highlighted the importance of physical factors at the proximal tubule in the response to saline infusion (Reineck et al., 1985). Reduction of the filtration fraction would thus have the effect of inhibiting fractional sodium reabsorption. Renal blood flow was not measured in this study. However, it has tended to remain stable during saline infusion in other studies, although redistribution of intrarenal blood flow could not be excluded (Alexander et al., 1974; McClanahan et al., 1985). The physical forces can however modulate proximal tubule reabsorption after saline infusion, even in the absence of changes in GFR and RBF. The mechanism is by alteration in the peritubular capillary oncotic pressure and disruption of glomerular tubular

balance, produced by haemodilution and the marked reduction in systemic plasma oncotic pressure.

During chronic oral salt loading, Oates et al. (1979) noted in man that the dopamine response preceded natriuresis, suggesting an effector role for dopamine. During intravenous infusion of saline, an acute dopamine response is demonstrable. However, in this setting, Faucheux and colleagues (1977) showed in rats, that the rise in dopamine succeeded the peak natriuretic response. In dogs, the urine dopamine response is appreciable and McClanahan et al. (1985) measured nearly a 4-fold increase in dopamine output. In man, however, the increase was only of the order of 28% in the study reported by Alexander and colleagues (1974). In the present study, dopamine excretion gradually increased and reached peak response one hour after the infusion, the same time as the peak sodium response. The increase was 26% on average, comparable to that reported, and values remained elevated at the end of the study.

To study the influence of endogenous dopamine on the natriuretic response to saline infusion, blockade of the effects of dopamine can be approached in two ways. Firstly, dopamine receptor antagonists can be used. It is likely that the DA_1 receptor mediates the natriuretic effects of exogenous dopamine so that a DA_1 receptor antagonist would be the suitable agent. The second approach would be to inhibit the renal synthesis of dopamine. The dopa decarboxylase inhibitor, carbidopa, was shown in the previous chapter to inhibit markedly the urinary excretion of dopamine, presumably reflecting a reduction in renal synthesis. Carbidopa was administered in two 100 mg doses during the study. It resulted in an appreciable inhibition of dopamine excretion and abolished the rise in response to saline. It is of note that dopamine levels were starting to recover at the end of the study, presumably reflecting a wearing-off of the inhibition of dopa decarboxylase.

Analysis of sodium excretion showed that the blockade of dopamine synthesis in no way affected the natriuretic response to saline infusion. At each time point, levels were matched and there was no significant difference in the cumulative salt outputs on carbidopa or placebo. It is also of note that carbidopa did not affect baseline natriuresis, suggesting that dopamine was not exerting an influence on sodium excretion under resting conditions. Similarly, dopa decarboxylase inhibition did not influence systemic blood pressure and pulse, or creatinine clearance, either during the baseline or after saline. The almost identical PRA values on carbidopa and placebo argue against a controlling role for dopamine on renin release under the conditions of this study.

These results need to be assessed with regard to previous studies in animals and man which have sought to assess a dopaminergic contribution to natriuresis. In anaesthetised rats the non-selective dopamine antagonist cis-flupenthixol partially attenuated natriuresis induced by Ringer loading (Pelayo et al., 1983). Similarly, racemic sulpiride inhibited saline-induced diuresis in rats, whereas the other antipsychotic drugs haloperidol, clozapine, pimozide and chlorpromazine were without effect (Imondi et al., 1979). In conscious dogs, carbidopa, administered by nasogastric tube at 1 mg/kg 8 hourly for 24 hours before saline infusion, markedly reduced dopamine excretion and abolished the large rise following saline in control animals. Neither saline nor carbidopa affected mean arterial pressure, GFR or RBF, but sodium excretion was reduced from a maximal value of 855 to 449 $\mu\text{eq}/\text{min}$, suggesting interference with tubular dopaminergic mechanisms (Sowers et al., 1984). Goldberg's group followed up this study and assessed the highly specific DA_1 and DA_2 receptor antagonists, SCH 23390 and domperidone respectively, in conscious dogs. Interestingly, there was no tendency towards attenuation of the natriuretic

response to saline infusion with either agent (Bradley et al., 1986b).

Krishna et al. (1985) reported in normal man, that the relatively non-specific dopamine antagonist, metoclopramide, markedly impaired sodium excretion in response to 2 litres of isotonic saline infused over 4 hours. In fact, no significant natriuretic response was apparent in the presence of the antagonist. Coruzzi et al. (1987) studied natriuresis during central hypervolemia induced by water immersion. In a normotensive control group, metoclopramide significantly blunted sodium excretion and it abolished exaggerated natriuresis demonstrated in a group of essential hypertensives. Further clouding the picture are studies reported by Bennett et al. (1982) and Coruzzi et al. (1986). Volume expansion was achieved in man by lower body pneumatic cuff and water immersion respectively. In both models, the specific DA_2 antagonist domperidone blunted the increment in sodium excretion.

It is difficult to reconcile the outcome of all these reported studies. A number of factors are likely to influence the results. Anaesthesia and pre-existing state of salt balance will affect the degree of activation of the renin-angiotensin and sympathetic nervous systems. Salt balance may also influence dopamine responsiveness: salt depletion might be expected to lead to up-regulation of dopamine receptors. In fact in the studies referred to, and in the present study, animals and human subjects were salt replete. Confusion may result from studies using antagonists that are not specific for dopamine receptors. Metoclopramide in particular, has multiple actions, including release of noradrenaline and cholinergic properties (Kuchel et al., 1985). Where highly specific antagonists were used, as in the study of Bradley et al. (1986), no apparent reduction in natriuresis could be demonstrated. It could be argued that the antagonists did not gain sufficient access to the dopamine receptors *in vivo*. However, the doses used had been shown to be effective in blocking

pharmacological effects of dopamine. Differences in animal model may be significant. Whereas the dopamine response to volume expansion is only modest in man, it is very much more marked in dogs, and dopamine may assume greater importance in this species. It is possible, in spite of appreciable suppression of output by carbidopa in the present study, that sufficient endogenous dopamine was present to modulate sodium excretion. However, the dose used had been previously shown to block the effects of gludopa, and a degree of attenuation might have been expected. Chronic inhibition of dopa decarboxylase, over three days, has been shown to result in a significant reduction in sodium excretion in man (Ball and Lee, 1977). It is important to note, in the present study, that dopamine excretion increased after the infusion and remained elevated at the end of the study, at a time when more than 70% of the sodium load remained to be excreted. It is conceivable that dopamine assumes greater significance as an endogenous natriuretic hormone during the later stages of the response, and it would be illuminating, though complicated in execution, to extend such a study over a period of 24 hours or more.

As discussed, the renal DA_1 receptor is believed to subserve the natriuretic effects of dopamine. The DA_2 receptor modulates noradrenaline release from sympathetic nerves and may assume greater importance when sympathetic tone is high. Volume expansion is associated with a decline in sympathetic nerve activity and it is therefore difficult to explain the effects of the selective DA_2 antagonist domperidone. Further studies will be required to elucidate the mechanism(s) involved.

Dopamine and ANP have a rather similar spectrum of activity. Both produce renal vasodilatation and are believed to have direct effects on the nephron. In addition, both are associated with inhibition of aldosterone release. A number of studies suggest that intact renal dopaminergic mechanisms are

necessary for the renal actions of infused ANP. The evidence is most persuasive in the rat model, where various dopamine antagonists including haloperidol and chlorpromazine (Marin-Grez et al., 1985; Pettersson et al., 1986), metoclopramide (Racz et al., 1986), and SCH 23390 and R-sulpiride (Webb et al., 1986), attenuated the natriuresis produced by atrial peptides. Most workers have attributed the effect to activation of DA₁ receptors, either directly or indirectly. In the dog however, the DA₁ antagonist SCH 23390 was without effect (Murphy et al., 1986). In man, metoclopramide did not affect the natriuresis and inhibition of renin release produced by infusion of h- α ANP (Jungmann et al., 1986). Of interest, in one study in man, carbidopa significantly reduced the natriuretic response to h- α ANP suggesting that dopamine synthesis and activity are mandatory for the effects of ANP (Wilkins et al., 1986). In the most recent study reported from this department, the selective DA₁ antagonist R-sulpiride had no effect on the renal response to low dose h- α ANP infusion in man (Freestone et al., 1988).

Species differences may partly account for the discrepant results, and in addition, the animal work has been mostly performed in the anaesthetised state. It is not clear how dopamine and ANP would interact in the kidney. Dopamine is believed to act through cAMP release after DA₁ receptor activation, whereas ANP appears to use cGMP as second messenger. Whereas dopamine has its principle tubular action proximally, there is little evidence that ANP exerts a direct proximal effect. Further studies, directed at cellular and biochemical level will be required to solve this problem.

In the present study, intravenous salt loading produced a marked increase in ANP that paralleled the natriuresis, showing a peak at the end of the infusion before declining, though remaining above baseline values. As discussed, evidence has been presented to suggest that ANP release is a

contributory factor in the natriuresis of volume expansion, but studies are dogged by lack of available antagonists.

A confounding factor in the assessment of components of natriuretic responses is that blockade of one component may be associated with compensatory upgrading of other components. There is no evidence in the present study that ANP responses were greater in the presence of dopamine blockade. In fact the peak response with carbidopa tended to be less than that with placebo, though this diminution was not significant. A lower response may be compatible with data presented by Racz et al. (1986). In rats, metoclopramide decreased both the baseline and saline-induced increase in plasma ANP, suggesting that dopamine normally exerts a tonic influence on ANP secretion. However, this has to be reconciled with the fact that in man, dopamine infusion was not associated with any change in plasma ANP levels (Shenker et al., 1987).

To analyse further the mechanisms of natriuresis and define the importance of respective nephron segments, the lithium clearance technique has been utilised in the setting of acute intra vascular expansion and the ensuing natriuresis. In studies reported in man, fractional lithium clearance increased by an order of 30% or so (Holstein-Rathlou et al., 1985; Atherton et al., 1987). This implies that the tubular rejection of fluid from the proximal tubule is increased, which may result from changes in the Starling forces at the proximal tubule, either due to alteration in the filtration fraction and/or reduction in plasma protein and hence peritubular capillary oncotic pressure, or due to direct hormonal effects. In the present study, fractional lithium clearance increased by 35% during saline infusion, a change that was highly significant. Lithium clearance represents the isotonic fluid volume leaving the proximal tubule. The ratio of sodium and lithium clearances therefore

represents the fraction of fluid entering the "distal tubule" (comprising the loop of Henlé, distal convoluted tubule and collecting duct), which escapes reabsorption. In response to intravenous saline, this ratio increased. However, absolute reabsorption by the distal tubule also markedly increased indicating that this segment partially compensated for the increased delivery to maintain glomerular tubular balance. Therefore the distal segment did not contribute to the natriuretic response under the conditions of the present study. These results are in agreement with those previously reported (Holstein-Rathlou et al., 1985; Nielsen et al., 1987).

However, it must be questioned whether lithium provided a suitable marker for tubular sodium handling. A dose of 1000 mg is somewhat higher than that usually used, but the plasma levels at the start of our study were below usual therapeutic levels so that a toxic effect cannot be invoked. Lithium showed a tendency towards increasing baseline sodium excretion, and PRA values were significantly elevated at the commencement of the study. Thereafter, the natriuretic response to saline infusion was reduced at each time point and cumulative sodium excretion above the control values was significantly reduced by around 40%. It could be argued that the kidney possesses a maximal capacity to excrete sodium after isotonic volume expansion which was attained in the present study. The apparent reduction in cumulative sodium excretion above control would thus be explicable purely in terms of the elevated baseline value, and not due to any specific effect of the lithium ion. However, this is unlikely to be the case. Absolute sodium excretion was reduced at each time point after the first hour of infusion, and a clear cross-over effect was apparent. More important evidence comes from the work of Epstein and colleagues (1979), in normal man. This group demonstrated that elevation of baseline sodium excretion by dietary supplementation markedly

exaggerated the absolute natriuretic response to volume expansion achieved by head-out water immersion. Thus in the present study, the elevated baseline sodium excretion induced by lithium might have been expected to increase, rather than decrease, the natriuretic response to volume loading. The most likely interpretation of the present results is that the lithium ion in some way interferes with normal excretory responses in the face of an intravenous salt load. However, the attenuation of natriuresis is unlikely to have involved dopaminergic mechanisms. Lithium did not interfere with dopamine output either during the baseline period or the infusion of saline. Also, as shown, blockade of dopamine synthesis did not attenuate the natriuretic response. The mechanism is therefore unknown, but this important interaction requires further study in two regards. Firstly, lithium must not influence renal function to be of use as a marker: a dose must be identified that is truly inert. Secondly, analysis of the effects of lithium on the nephron may advance our knowledge of receptor and transport mechanisms, and intracellular processes involved in sodium transport and renal hormonal release.

CHAPTER SIX

FINAL DISCUSSION AND CONCLUSIONS

The diverse experimental approaches into the actions of dopamine in the periphery have demonstrated the amine to possess various interesting features. By activation of distinct receptors it is capable of producing systemic and renal vasodilatation and of modulating sympatho-adrenal outflow. By a combination of haemodynamic and direct tubular effects it is actively natriuretic, and influences hormonal release from the kidney and adrenal cortex.

As a local regulator of sympathetic activity at the sympathetic ganglion and the pre-synaptic DA_2 receptor, it may function as a classical neurotransmitter. A different situation appears to pertain to the kidney, and conceivably the adrenal cortex, where large amounts of dopamine are produced extraneuronally, and where it may act as a local hormone. Such a dichotomy of function is not unique: there is a growing appreciation that regulatory peptides can be produced by endocrine and neural tissue, and function, individually or in combination, as circulating hormones, local regulators and neurotransmitters (Polak and Bloom, 1983).

Research interest into dopamine in the periphery, and especially the kidney, has focussed on three main areas; a potential physiological role in blood pressure regulation, hormonal interaction and renal salt balance; involvement of dopaminergic mechanisms in pathophysiological conditions, notably essential hypertension; and the pharmacological manipulation of dopamine receptors in the treatment of various disease states. The work in this thesis has touched upon some of these aspects in the kidney in normal man.

A physiological function in the kidney is strongly suggested by the production and excretion of large amounts of free dopamine, by the close relationship between dopamine excretion and sodium output in the urine, and by the characterisation of a widespread distribution of dopamine receptors. Such observations do not however, prove that endogenous dopamine has physiological

significance and more compelling evidence accrues when an inhibitor of dopamine can be shown to alter some parameter of normal function. Such studies have been approached using either dopamine receptor antagonists or dopa decarboxylase inhibitors to block dopamine synthesis, and a number of animal models have been utilised, notably in man, dog and rat.

As yet, no firm consensus has emerged, and it must be conceded that many studies have failed to demonstrate significant effects. Certainly, in the present work, the effective blockade of renal dopamine synthesis with carbidopa, did not consistently influence sodium excretion or hormonal release, either under baseline conditions or after acute intravenous salt loading. There are, however, a number of points which must be raised before an important role for endogenous dopamine in renal function can be discounted.

Analytical methods for the study of renal function in vivo are crude. The measurement of indices in blood and urine represents an "averaging-out" of the outcome of a host of interacting physical, hormonal and neuronal influences at the cellular microenvironment in compartmentalised regions of the kidney. Salt balance is subject to tight and complex control mechanisms, and physiological, as opposed to pharmacological, manipulations, may produce subtle, but nevertheless real changes in function which are not easily demonstrable in short term, in-vivo, studies. Work in man, on an out-patient basis is particularly difficult in this setting as subjects are often not in true steady state for salt balance, individual variation may be large, and various extraneous influences unaccounted for. In addition, compensatory adjustments in other control systems may mask the significance of changes in any particular system. In vitro work allows characterisation of isolated stimuli, but the very principle negates the physiological implications.

The development of highly specific and selective dopamine receptor

antagonists may clarify certain issues. Many studies should be repeated with the newer agents, as the earlier antagonists used crossed the blood-brain barrier and effects were likely to be complex due to their non-selective nature. However, even here, caution is required. A particular antagonist, active in an *in vitro*-model, or capable of nullifying the effects of administered dopamine, may still not possess a suitable pharmacokinetic profile to allow access to dopamine receptors in sufficient concentrations *in vivo*. If endogenous dopamine is a physiological mediator it is important to define the necessary conditions by which such a role can be demonstrated, and these are not yet clear. With the known natriuretic action of the administered amine, it might be expected that dopamine is involved in the response to excess sodium intake. Both oral salt loading and intravenous saline volume expansion stimulate dopamine excretion, suggesting a homeostatic function. However, the response in man is modest. As discussed earlier from an evolutionary standpoint, if the dopaminergic mechanism was developed to offset excessive sodium retention, man, with principally a herbivorous pedigree, might not be the most suitable model. The poor dopaminergic responsiveness displayed in blacks, from limited work performed, has been alluded to. The greater responsiveness demonstrable in dogs, with a carnivorous background, may give credence to this theory, but direct comparisons between species have not been undertaken.

Aldosterone secretion appears to be under tonic maximal dopaminergic inhibition under salt replete conditions, and this inhibition can be removed by salt depletion. Such observations also point to a physiological role in the response to salt excess, where inhibition of aldosterone would have a permissive action in natriuresis.

Mannelli and Pupilli (1988) have recently however produced interesting

data in man to suggest that dopamine is important in the catecholamine response to stress. Domperidone, the DA_2 antagonist, significantly increased catecholamine excretion during graded exercise, though having no effect at rest. Thus endogenous dopamine appeared to be modulating sympatho-adrenal outflow. The sympathetic nervous system is activated by salt depletion, and exerts salt-retaining and vasoconstrictive actions. It is tempting to speculate that under such conditions dopamine prevents excessive vasoconstriction and renal shut-down, in a manner rather analogous to that of the renal prostaglandins.

There is no evidence as yet, that dopamine functions as a circulating hormone under normal circumstances: plasma levels of free hormone are extremely low and it seems likely that activation of receptors results from local synthesis at tissue level, either intra- or extra-neuronally. The infusion of dopamine itself is unlikely to provide useful information on physiological function for two main reasons. Firstly, infusion, even at small doses, enormously increases plasma dopamine levels. Secondly, its ability to stimulate α and β receptors makes interpretation of results difficult. The many studies performed with infused dopamine have, in some ways, clouded our understanding of dopaminergic mechanisms. In addition, the propensity for systemically administered dopamine to produce peripheral vasodilatation and reduce blood pressure complicates the renal actions in that various compensatory mechanisms, including the renin-angiotensin and sympathetic nervous systems, are activated by the resulting systemic changes.

Gludopa has proved a valuable agent in investigating the action of dopamine in the kidney as divorced from those in the systemic circulation. The large increase in urine dopamine excretion, with only modest elevation of plasma dopamine, is paralleled by its renal actions without marked alteration in

blood pressure and pulse, reflecting the renal specificity of the pro-drug. The levels of urine dopamine achieved by gludopa are well above the physiological range. The important point, however, is that dopamine synthesis occurs at tubular level, mimicking the normal pattern of production. Therefore, observations made with gludopa may be pertinent to the physiological state. It might be expected that such large tissue concentrations of dopamine in the kidney would activate α and β , as well as dopamine receptors, and this possibility has not been excluded. However, the pattern of action, vasodilatation and natriuresis, and blockade of effects by dopamine antagonists, points to dopamine receptor activation being predominant.

It can be concluded for the present studies and from other reported work that dopamine exerts independent action in the kidney, without the requisite mediation of other hormonal, neural or physical factors. More likely is a system of parallel influences, including dopamine, interacting in an array of negative and positive feedback loops, forming a highly complex control mechanism which maintains homeostasis and is able to respond rapidly to changes in the local and wider environment.

The clear inhibition of PRA produced by gludopa is an observation of theoretical and potential therapeutic significance. It is conceivable, that under conditions of salt excess, inhibition of renin by dopamine would amplify the direct suppression of aldosterone at the adrenal cortex, the result being to allow effective natriuresis. However, as yet, dopamine antagonists have not produced consistent effects on renin release and the question needs further study.

Although the initial studies concerning dopamine in the kidney were performed more than 25 years ago, fundamental questions remain, in particular the relationship to prevailing salt status. By some unknown mechanism, a

change in salt content alters dopamine synthesis and release. In vitro evidence suggests that the uptake of L-dopa into proximal tubular cells is a sodium dependant process. It is not known whether control of dopamine synthesis lies at the level of cellular dopa decarboxylase, uptake and transfer of L-dopa into the cell, or depends on the availability of L-dopa at the proximal tubule. This last point raises questions as to the source and regulation of L-dopa in plasma, and thence the filtered load, under conditions of altering salt status.

Little is known of other physiological or pharmacological manoeuvres which influence dopamine synthesis and excretion. Frusemide has proved to be an effective stimulus but it remains unclear exactly which aspect of its action is responsible. Further studies should assess whether an increase in GFR per se, increasing the filtered load of sodium and L-dopa, provides the stimulus or whether alterations in renal haemodynamics and physical forces at the proximal tubule are pre-requisite. The effects of calcium antagonists on dopamine synthesis are unknown. In addition to producing renal vasodilatation and natriuresis, their use may indicate whether the intracellular events that modulate dopamine synthesis are dependant on the calcium second messenger system.

The fortuitious discovery that lithium blocked the natriuresis induced by gludopa should provide new insights into the mechanism of action of dopamine. It is firstly important to determine whether renal vasodilatation produced by dopamine is similarly attenuated by lithium - the use of gludopa will allow renal events to be more easily distinguished. Measurement of urinary cAMP has not been useful in studying interactions between dopamine and lithium in the kidney in man. It may be more appropriate to use an animal model such as the rat and measure renal tissue cAMP levels after pharmacological intervention. Interference with cAMP production after dopamine stimulation would suggest

that the action of lithium is on the DA_1 receptor. Confirmatory evidence of this could be sought by assessing the effect of lithium on the renal actions of specific DA_1 and DA_2 agonists. The action of lithium may however be rather complex. Its attenuation of natriuresis after intravenous saline did not appear to involve a dopaminergic mechanism. In addition, it has recently been demonstrated in man to block the natriuretic action of ANP, widely believed to be a cGMP-mediated phenomenon (Freestone, personal communication). These observations suggest that it may be a general inhibitor of stimulated sodium excretion, acting at some common pathway after receptor activation by diverse stimuli.

In spite of the arguments concerning a physiological role for endogenous dopamine, it clearly exerts potent pharmacological actions after administration. Dopamine itself has drawbacks that limit its therapeutic potential, namely its propensity to stimulate α and β adrenergic receptors, and its poor oral bioavailability, and attempts have been made to produce pharmacological agents which overcome these problems. Thus fenoldopam and bromocriptine are orally available agonists, active in man, at DA_1 and DA_2 receptors, respectively (Goldberg, 1988). A different approach has been to develop prodrugs which avoid metabolism before absorption and release free dopamine into the circulation. The dopamine precursor L-dopa has already been mentioned. Recently, the pharmacological properties of TA-8704 (N-(N-acetyl-L-methionyl)-O, O-bis(ethoxycarbonyl) dopamine) have been evaluated. Free dopamine, released into plasma after oral administration, correlated with the increment in RBF in dogs, and it produced an increase in RBF and sodium excretion in human subjects with renal impairment (Nishiyama et al., 1985; Ozawa and Mort, 1985). Ibopamine is the diisobutyric ester of N-methyl dopamine (epinine). After oral

ingestion it is converted by plasma esterases to epinine which is active on DA₁ and to some extent α and β_2 receptors (Goldberg, 1988). Stimulation of dopamine receptors in the systemic circulation readily produces generalised vasodilatation and reduction in afterload, and the various agents are being evaluated for possible therapeutic use in essential hypertension and cardiac failure. The ideal agent would be orally active, and preferably not cross the blood-brain barrier. A combination of DA₁ and DA₂ agonist activities would seem beneficial - DA₁ activity to produce direct vasodilatation and enough DA₂ activity, to avoid nausea on the one hand, but block baroreceptor - mediated sympathetic activation and direct renin stimulation, on the other. An alternative would be to combine a DA₁ agonist with an inhibitor of angiotensin converting enzyme, for a possible synergistic action.

Two further novel approaches to the manipulation of dopamine receptors have been reported, Langer et al. (1987) demonstrated in cat atria that the mono-amine oxidase inhibitor pargyline increased endogenous levels of dopamine, and postulated that increased pre-synaptic DA₂ activity might contribute to the antihypertensive effect of this drug group. Berkowitz and Ohlstein (1987) have studied the dopamine β hydroxylase inhibitor SKF 102698. In spontaneously hypertensive rats it produced a marked increase in vascular dopamine/noradrenaline ratio, concurrent with a fall in blood pressure.

Whether or not the kidney has direct pathophysiological significance in the genesis of essential hypertension, it is a major determinant of the response to antihypertensive therapy. Active systemic vasoconstriction is a characteristic feature of the hypertensive state, and it might be expected that vasodilatation would constitute an ideal treatment to reverse the abnormality. However the classical vasodilators minoxidil, hydralazine and sodium nitroprusside are not effective as monotherapy. The initial reduction in system-

ic vascular resistance and blood pressure activates baroreceptor-mediated sympathetic drive and stimulation of the renin-angiotensin system, and the renal response is to produce sodium retention - effects that offset drug action (Hollenberg, 1986).

Guyton et al., (1974) propounded the concept of the renal function curve in which blood pressure is primarily dictated by renal excretory function. According to this model, a causal approach to the treatment of hypertension would be enhancement of the renal capacity to excrete sodium. Struyker Boudier (1980) developed a computer simulation model which predicted that the consequences of preferential renal vasodilatation would represent a reversal of the autoregulatory hypothesis for essential hypertension (Guyton et al., 1974). Thus renal vasodilatation and natriuresis lead to reduction in intravascular blood and ECF volume, and hence cardiac output and tissue perfusion. Reduced perfusion would be opposed by local autoregulatory mechanisms resulting in vasodilatation and a shifting of the renal function curve to a lower blood pressure. In this light Nievelstein (1987) has made interesting observations in the spontaneously hypertensive rat. He compared the hydralazine-like compound CGP 18137A with its N-acetyl-L- γ -glutamated derivative CGP 22979A. Whereas the former produced a response pattern similar to that of the classical arteriolar vasodilators, the response for the latter was very different. An initial natriuresis and elevation of RBF was followed by a gradual reduction in mean arterial pressure over 5 days without elevation of heart rate or stimulation of renin release. There are direct parallels here with gludopa. CGP 22979A is a prodrug of the parent compound which produces high concentrations of drug in the kidney because hydrolysis by acylase and γ -glutamyl transferase occurs at a higher rate in the kidney than elsewhere.

MacDonald et al. (1988) have recently infused gludopa into normal

volunteers for 8 hours. Natriuresis persisted for 6 hours, there was a small reduction in blood pressure, and compared to placebo, no increase in PRA. Such infusions need to be performed over a number of days and in a group of essential hypertensives. According to the computer model, blood pressure might be expected to decline gradually without reactive increase in PRA. In comparison with CGP 22979A, gludopa may possess two further beneficial properties. The first is its ability to directly inhibit renin release. Secondly, if it is the case that a defect of dopamine mobilisation exists, at least in a proportion of those with essential hypertension, the use of gludopa may reverse a specific defect in the aetiology of the condition.

By having effects targeted at the kidney, without exerting systemic actions or incurring compensatory negating responses, gludopa may have a number of potential therapeutic actions. The agent should be assessed in the setting of incipient and established acute renal failure in which dopamine itself has been applied. It may be of benefit in cadaveric renal transplantation both in the donor, before organ removal, and in the early post-operative period. Particularly it would be of interest to determine whether gludopa can increase the incidence of immediate function, and, by counteracting nephrotoxicity produced by the standard immunosuppressive agent cyclosporin, allow maximal effective dosage to be administered. Similarly its use may encourage renal function after liver and cardiac transplantation. The common tendency towards renal impairment in acute hepatic failure may be prevented by encouraging renal dopamine production with gludopa. The agent is at present being evaluated in women with ovarian carcinoma under treatment with cis-platinum, a drug whose benefits and maximal dose prescribable are limited by nephrotoxicity (Lee, personal communication).

Parental gludopa possesses a theoretical advantage over dopamine in

that it can safely be given through a peripheral vein, removing the need for central access. As a prodrug, it is unlikely to produce the local vasoconstriction and tissue sloughing, due to α -agonist activity, that is associated with extravasation of the parent compound.

At present, the use of gludopa in chronic conditions such as essential hypertension is limited by its requirement for parenteral administration. Research must be directed towards producing oral congeners which avoid metabolism during drug absorption. It must then be assessed in those conditions in which renal vasodilatation and the promotion of natriuresis might be advantageous. For instance, a possible application might be to determine whether the progressive deterioration of function in chronic renal failure can be altered. The combination of gludopa with a systemic vasodilator or inotropic agent may provide a beneficial spectrum of activity in the treatment of congestive cardiac failure.

With the increasing interest in dopamine and the kidney it seems likely that the next decade will see a focusing of the physiological and pathophysiological significance of endogenous dopamine, and it is quite possible that pharmacological manipulation of dopamine receptors will have added to our therapeutic armamentarium.

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Papers

resulting from work in this thesis.

The effect of intravenous frusemide on urine dopamine in normal volunteers+ Studies with indomethacin and carbidopa. R.F. Jeffrey, T.M. MacDonald, M.K. Rutter, S. Freestone, J. Brown, R.R. Samson and M.R. Lee. Clinical Science, 1987, 73, 151-157.

A comparison of the renal actions of γ -L-glutamyl-L-dopa and γ -L-glutamyl-L-tyrosine in normal man. R.F. Jeffrey, T.M. MacDonald and M.R. Lee. Clinical Science, 1988, 74, 37-40.

The effect of carbidopa and indomethacin on the renal response to γ -L-glutamyl-L-dopa in normal man. R.F. Jeffrey, T.M. MacDonald and M.R. Lee. British Journal of Clinical Pharmacology, 1988, 25, 195-201.

The effect of lithium on the renal response to the dopamine prodrug gludopa in normal man. R.F. Jeffrey, T.M. MacDonald, J. Brown, P.W.H. Rae and M.R. Lee. British Journal of Clinical Pharmacology, 1988, 25, 725-732.

The effect of carbidopa and lithium on the systemic and renal response to acute intravenous saline loading in normal man. R.F. Jeffrey, T.M. MacDonald, S. Freestone and M.R. Lee. Accepted for publication. Nephrology, Dialysis and Transplantation.

Presentations to Scientific Meetings.

Urinary dopamine - sodium relationships under basal and frusemide - stimulated states. R.F. Jeffrey, T.M. MacDonald, J.A.J.H. Critchley and M.R. Lee. Scottish Society for Experimental Medicine, 6th June, 1986.

Failure of indomethacin to prevent the increased urine dopamine levels produced by intravenous frusemide in normal volunteers. R.F. Jeffrey, T.M. MacDonald, M.K. Rutter, S. Freestone, J. Brown, R.R. Samson and M.R. Lee. Medical Research Society Meeting at Trinity College, Dublin, 24th-25th July, 1986.

Studies with γ -glutamyl-L-dopa and γ -glutamyl-L-tyrosine in man: Relationships to intrarenal dopamine production and natriuresis. R.F. Jeffrey, T.M. MacDonald, J. Brown and M.R. Lee. Satellite Symposium of the 11th Scientific Meeting of the International Society of Hypertension, Gent, Belgium, 7th-8th September, 1986.

The renal response to gludopa in man is attenuated by carbidopa but not by indomethacin. R.F. Jeffrey, T.M. MacDonald and M.R. Lee. Third European Meeting on Hypertension, Milan, 14th-17th June, 1987.

Lithium attenuates the renal response to γ -L-glutamyl-L-dopa in man. R.F. Jeffrey, T.M. MacDonald and M.R. Lee. British Pharmacological Society Meeting, Oxford, 9th-11th September, 1987.

Lithium but not carbidopa attenuates the natriuretic response to intravenous salt loading in normal man. R.F. Jeffrey, T.M. MacDonald and M.R. Lee. Proceedings of the XXVth Congress of the European Dialysis and Transplant Association - European Renal Association, 5th-8th September, 1988.